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THE EFFECT OF INORGANIC SALTS ON CALCIFICATION[1]

A Preliminary Note

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Studies of the process of calcification of cartilage and bone *in vitro* have shown that substances other than calcium and phosphorus affect the deposition of tertiary calcium phosphate in those tissues. For example, calcification is inhibited or delayed by proteins, or colloids, such as gum acacia.

These studies have been continued in an attempt to determine what influence, if any, is exerted on calcification by individual inorganic salts. Sodium chloride was chosen as the first of these compounds to be studied. Solutions were prepared containing calcium, potassium, magnesium, carbonate, phosphate and sulphate in quantities analogous to those found by analysis in normal blood serum. The basal solutions contained 32 millimols of sodium (added as sodium bicarbonate) and no chlorine. Sodium chloride was added to samples of these basal solutions in varying amounts. The reaction of these samples was adjusted in all cases to approximately pH 7.3 by means of carbon dioxide, and slices of bone and cartilage free from lime salts were kept in them for 19 or 20 hours at a temperature of 37.5°C.

The addition of small amounts of sodium chloride to the basal solutions did not interfere in any way with the deposition of calcium salts in the tissue. When, however, this salt was added in sufficient amounts to increase the concentration of sodium to about 75 millimols, (the chlorine concentration in this event would be about 43 millimols) calcification was definitely inhibited. No lime salts were deposited from such a solution during periods as long as five days.

Since the concentration of sodium in the blood serum is approximately 140 to 154 millimols, and that of chlorine is from 95 to 105 millimols, it is evident that sodium and chlorine ions in a much lower

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concentration than that in which they are found in the blood stream prove an effective barrier against rapid calcification. It is equally obvious that, even apart from the inhibitory influence exerted by proteins and other colloids, rapid deposition of calcium salts from the circulating blood is impossible by reason of the NaCl concentration—other things being equal.

Further studies on NaCl and other salts of different valence types are being carried on with the hope of determining among other things the nature of this inhibiting salt effect.

A BIOMETRIC STUDY OF THE RELATIVE CELL VOLUME OF HUMAN BLOOD, IN NORMAL AND TUBERCULOUS MALES[1,2]

RAYMOND PEARL AND JOHN RICE MINER

I

For some time Dr. B. H. Schlomovitz (1), Director of the Clinical Research Laboratories of the National Military Hospital, National Home, at Milwaukee, has been interested in the relative cell volume of the blood, in relation particularly to tuberculosis. He has collected a considerable body of statistical material on the subject, using great care to ensure accuracy in the original measurements. This statistical material Dr. Schlomovitz has very kindly turned over to us for analysis, with the results set forth in this paper.

We were chiefly interested in undertaking this piece of biometric work because it offered an opportunity to add to the collection of data in this Institute having to do with the normal age changes in structural and physiological characters of the organism. A comprehensive study of the biology of senescence and natural death is a main feature of our research program. In spite of the large literature which exists on these subjects, there is an almost complete lack of precise, quantitative information as to the somatic changes associated with advancing age. Whenever possible, we are trying to add to this meager store.

The material turned over to us by Dr. Schlomovitz comprises three series of observations, differentiated in the following manner:

Group I consists of 272 normal males, ranging in age from 20 years to 49 years.

Group II consists of 449 men, in hospital because of active tuberculosis of the lungs. They range in age from 21 years to 48 years.

Group III consists of 50 men, who, having been hospitalized for

[1] From the Institute for Biological Research of the Johns Hopkins University. This paper is based upon records collected by Dr. B. H. Schlomovitz.

[2] Received for publication, October 25, 1926.

active pulmonary tuberculosis, were, at the time of record, about to be discharged as arrested or healed cases. They range in age from 25 years to 40 years.

While neither the number of persons, nor the range of age, are as extensive as could be desired, nevertheless, the material appeared worthy of careful analysis, because of its accuracy within its scope, and because the relative cell volume of the blood is a significant physiological character of the organism.

For each individual the following items were recorded.

a. Age in years.

b. Body-weight in pounds.

c. Stature in inches.

d. Percentage volume of the cellular elements of the blood. This datum is sometimes referred to as the relative "red cell volume." As a matter of fact, of course, all the cellular elements of the blood are included in the determination. While the red cells do in fact make up the overwhelming bulk of the recorded figure, it seems desirable to use the term "cell volume." We note that Henderson (2) uses this designation in his latest discussion of the physiology of the blood.

Throughout the paper the letters *a*, *b*, *c*, and *d* will be used as subscript letters, and generally in equations, to denote the characters against which they stand in the above list.

The normal males of Group I were derived from four sources, as follows:

1. Physicians and technicians with a record of no major illnesses in the previous three years, and a practically continuous record of daily application to work.

2. Other hospital aids and helpers with the same qualifications.

3. Mechanics and office help selected by the foremen of respective departments in two large manufacturing establishments with no record of illness by the first aid hospital unit attached to the organization. Many of these had worked five or more years as skilled laborers.

4. Men of Dr. Schlomovitz's personal acquaintance who were active in business life and had no record of illness for three or more years previous to taking the test.

The technique used to obtain relative cell volume in this material, was as follows: After inserting 0.4 cc. of a 1.6 per cent sodium oxalate

solution in the graduated tube of the Sahli hemometer there were added 2.0 cc. of actively circulating blood drawn from a vein of the elbow region. The blood was mixed thoroughly by inverting the tube three times, covering the open end with the type of flat, thin, rubber cap with rolled edges used for a vaccine vial or test tube and then centrifugating for half an hour or longer. The corpuscular deposit was subtracted from the total blood used, and the percentage volume calculated.

In the present paper we shall first discuss the normal series (Group I), and then consider the two tuberculous groups. The questions with which we shall here chiefly deal, on the basis of the material comprised in the sample of normal males, are:

1. What is normal relative cell volume of the blood in males, and to what extent does it vary among different individuals?

2. To what extent and in what way are differences between individuals in respect of the relative cell volume of the blood correlated with differences between the same individuals in respect of stature and body-weight?

3. To what extent and in what way are differences between individuals in respect of relative cell volume correlated with differences in age, among individuals of the same stature and body-weight?

4. What is the influence of pulmonary tuberculosis, active or arrested, upon the relative cell volume of the blood, when stature, age, and body-weight are constant.

II

The distribution of individual variation in the relative cell volume of the blood in the normal males of Group I is shown graphically in the histogram shown as Figure 1.

It is plain from Figure 1 that there is no strikingly unusual feature in the distribution of variation in relative cell volume. The histogram shows the usual "cocked-hat" shape, and is nearly symmetrical about the mean value, allowing for irregularities due to the comparatively small size of the sample.

In order to indicate the comparative variability of relative cell volume as compared with the other characters for which data are available in this group of men, Figure 2 has been prepared. This

diagram consists of four frequency polygons. As abscissa is plotted the relative percentage value of each of the four characters, relative cell volume, stature, body-weight, and age, by determining the percentage which each observed value is of the mean value for that character. Since all the characters are determined on the same group of individuals, and therefore the total frequency is the same for all, the ordinates are plotted in terms of absolute frequencies per one per cent of the mean of each character. In other words, if the class interval in one of the original distributions is 2.19 per cent of the mean, the ordinates in the frequency polygon equal the original frequencies

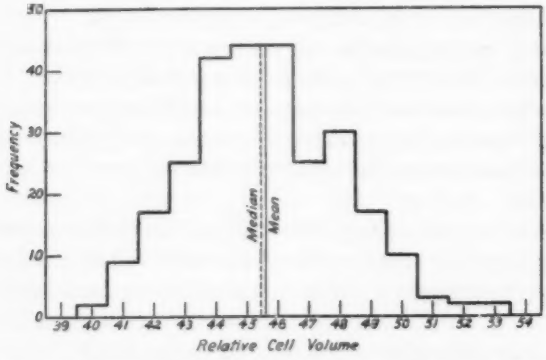

FIG. 1. HISTOGRAM SHOWING INDIVIDUAL VARIATION IN RELATIVE CELL VOLUME OF THE BLOOD IN 272 NORMAL MALES OF GROUP I

divided by 2.19, or the frequency in a class interval of one per cent. In this way the frequency polygons all have the same area.

This method of plotting superimposes the different polygons of variation on a common Cartesian coördinate grid, with the mean value for each of the compared variables at the same abscissal point. It constitutes no new method of measuring biological variation, but merely visualizes effectively what the coefficient of variation measures. We believe it to be a useful extension of the conventional biometric expository technique. It is more particularly discussed in another paper (3).

The method of plotting used in Figure 2 shows at a glance that

the 272 men differ among themselves far more widely in respect of age and body-weight than they do in respect of stature or relative cell volume. The variation polygon for stature shows the least scatter. That for relative cell volume is somewhat, but not greatly, more spread. Those for age and body-weight are wide, flat distributions, indicating a relatively high variation in the group in respect of these characters. The four basic descriptive constants, mean, median, standard

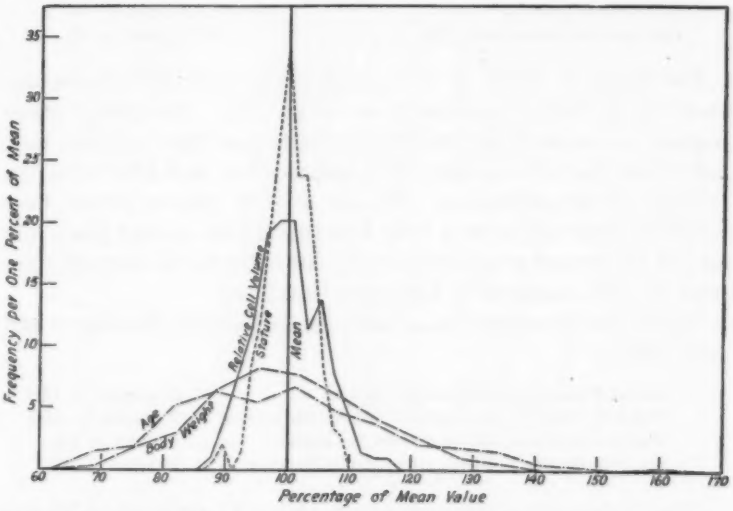

FIG. 2. SUPERIMPOSED VARIATION POLYGONS FOR (1) RELATIVE CELL VOLUME, (2) STATURE, (3) BODY-WEIGHT, AND (4) AGE, IN THE NORMAL MALES OF GROUP I

See text for further explanation

deviation, and coefficient of variation, for the characters *a*, *b*, *c*, and *d*, in Group I, are given in Table I, together with their probable errors.

From the data so far presented we note the following points regarding variation in this group of normal men, in respect of the four characters considered:

1. Having regard to the size of the sample dealt with, there appears to be no significant skewness or asymmetry in the variation of any of

the four characters. The median lies close to the mean in all four distributions.

2. It is of interest to compare the present values for body-weight and stature, taken about seven years after the end of the World War with the data of Davenport and Love (4), taken at the time of the war and at demobilization. These authors give values as follows:

Mean body-weight, accepted recruits of 1917–1918	= 141.54 pounds (p. 119)
Mean body-weight, whites, at demobilization, 1919	= 144.89 pounds (p. 119)
Mean stature, recruits	= 67.49 inches (p. 67)
Mean stature, at demobilization	= 67.72 inches (p. 67)

The excess in weight of the present group over these figures can probably be wholly accounted for on two grounds. The present group happens to consist of men averaging a little more than a half an inch taller than the recruits, and over a quarter of an inch taller than the veterans at demobilization. Because of their greater stature they would be expected to be a little heavier. In the second place, the men of the present group are several years older, on the average, than were the men measured by Davenport and Love.

Turning to the comparison of variation we find from Davenport and Love that:

Standard deviation, body-weight, recruits	= 17.42 pounds (p. 121)
Standard deviation, body-weight, demobilized men	= 17.06 pounds (p. 121)
Standard deviation, stature, first million recruits	= 2.71 inches (p. 73)
Standard deviation, stature, white demobilized men	= 1.69 inches (p. 73)

These figures are of the same general order of magnitude as the corresponding values in Table I. Considering the variation in relation to the mean values, it appears that the present sample probably does not significantly differ from the general population of American soldiers in the World War, in respect of variability.

3. Regarding the relative cell volume of the blood Starling (5) says (p. 729): "The average volume of the corpuscles in human blood can be taken as 50 per cent of the total amount, different estimations having given figures varying from 48 to 54 per cent." If the present group may be taken as a fair sample of normal, healthy men, which on all other grounds it seems to be, Starling's estimated figures would seem to be statistically a poor guess.

TABLE I

Variation constants for Group I

CHARACTER	MEAN	MEDIAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
a. Age.....	30.59 ± 0.21 years	30.23 ± 0.26 years	5.22 ± 0.15 years	17.06 ± 0.51 per cent
b. Body-weight....	151.56 ± 0.82 pounds	150.48 ± 1.03 pounds	19.95 ± 0.58 pounds	13.16 ± 0.39
c. Stature.....	68.13 ± 0.10 inches	68.10 ± 0.13 inches	2.45 ± 0.07 inches	3.60 ± 0.10
d. Relative cell volume.....	45.59 ± 0.10 per cent	45.43 ± 0.13 per cent	2.47 ± 0.07 per cent	5.42 ± 0.16

Gram (6) summarizes the results of three studies in cell volume in Table II (his Table VII).

TABLE II

Cell volume percentage (from Gram (6))

VALUES	GRAM AND NORGAARD (7) (HIRUDIN-BLOOD HEMATOKRIT)		GRAM (8) (9) (CITRATED BLOOD, LARGER TUBES)	
	Men	Women	Men	Women
Maximum	50.0	43.0	51	45
Minimum	42.4	38.8	43	36
Average	46.3	40.6	48	40
Subjects	10	10	25	96

TABLE III

Coefficients of variation for physiological characters in man. Data for males only, except where otherwise noted

CHARACTER	COEFFICIENT OF VARIATION	AUTHORITY
Steadiness of hand, females only	48.54–69.59	Tildesley (14)
Visual acuity	39.12	Holzinger (12)
Dermal sensitivity	35.70	Pearson (10)
Rapidity of hand, females only	23.91–29.49	Tildesley (14)
Strength of grip, all ages, left hand	26.85	Arthur (13)
Strength of grip, all ages, right hand	25.93	Arthur (13)
Swiftness of blow	19.4	Pearson (10)
Reaction time to sound	19.15	Harmon (11)
Reaction time to sight	19.02	Harmon (11)
Intelligence quotient, both sexes	18.01	Pearson (15)
Vital capacity	17.90	Holzinger (12)
Respiration rate per minute	17.80	Whiting (16)
Breathing capacity	16.6	Pearson (10)
Strength of grip, reduced to age 40.5, left hand	16.27	Arthur (13)
Auditory acuity	15.84	Holzinger (12)
Strength of grip, reduced to age 40.5, right hand	15.43	Arthur (13)
Strength of pull	15.0	Pearson (10)
Pulse rate per minute	14.89	Whiting (16)
Relative cell volume of the blood	*5.42*	*This paper*
Oral temperature	0.49	Whiting (16)

Comparing the mean values for men in the table with those of Table I, it is seen that Gram's values are higher in both cases. There

are a number of possibilities which might account for this difference. It may be due to systematic differences in technique, or it may be a result simply of the small size of Gram's series, statistically speaking, with the possibility of bias which such small samples always present. It may possibly represent some racial difference. With any information available to us, it is impossible to form a decisive judgment on the point. It is of interest to note in this connection that Gram gives the lower range end as 42.4 per cent in one series, and 43 per cent in the others. Table IV *infra* shows that 28 men out of the 272, or approximately 10 per cent of the total, in the present series gave values below 42.5 per cent. Similarly he gives 50 and 51 per cent as values for the upper range end. Table IV shows that there were 7 men in the present sample with values above 50.5 per cent.

4. With a coefficient of variation of 5.42 ± 0.16, relative cell volume shows an unexpectedly low relative variability for an essentially physiological characteristic. It compares with other coefficients of variability for physiological characters in man as shown in Table III.

While the list of physiological and psychical characters in Table III is by no means exhaustive, it is sufficient for the present purpose. The table shows at once that relative cell volume of the blood and oral temperature have a wholly different and much lower order of relative variability from individual to individual, than do the other characters listed.

5. It will be noted from the preceding paragraph that the relative variability of the two physiological characters, relative cell volume and body temperature, which are indices of the internal environment of the organism, is low. This suggests that not only is the internal environment of the same individual regulated to great constancy, as Henderson (2) has shown for the blood and circulation, but it is relatively more constant from individual to individual, under normal conditions, than are most morphological characteristics, not to mention other physiological and psychological attributes. Evolution has, in short, apparently produced a considerable degree of absolute constancy in the internal environment of the separate individuals of at least one species of animal, man.

III

We turn next to the normal correlations between relative cell volume and the other characters in which we have observations, age, body-weight, and stature. The zero order correlation tables concerned are shown in Tables IV, V, and VI.

It is evident from simple inspection of these tables that there is no close correlation between variations in relative cell volume, on the one

TABLE IV

Correlation of relative cell volume and age. Normal males of Group I

AGE	RELATIVE CELL VOLUME														
	39.5–40.4 per cent	40.5–41.4 per cent	41.5–42.4 per cent	42.5–43.4 per cent	43.5–44.4 per cent	44.5–45.4 per cent	45.5–46.4 per cent	46.5–47.4 per cent	47.5–48.4 per cent	48.5–49.4 per cent	49.5–50.4 per cent	50.5–51.4 per cent	51.5–52.4 per cent	52.5–53.4 per cent	Totals
20–21.9	–	–	–	1	3	1	1	–	2	1	–	–	–	–	9
22–23.9	–	–	–	–	3	2	1	1	2	2	–	–	1	–	12
24–25.9	–	1	1	4	3	7	4	5	3	3	2	1	–	–	34
26–27.9	–	1	1	5	11	5	7	4	2	–	1	2	1	1	41
28–29.9	–	2	1	4	5	3	7	2	7	2	2	–	–	–	35
30–31.9	1	1	5	3	6	6	9	1	7	4	–	–	–	1	44
32–33.9	–	1	1	2	3	7	7	6	1	1	2	–	–	–	31
34–35.9	–	2	2	2	3	6	4	1	2	1	1	–	–	–	24
36–37.9	1	–	5	–	2	2	1	1	1	–	2	–	–	–	15
38–39.9	–	1	1	4	–	1	1	2	1	1	–	–	–	–	12
40–41.9	–	–	–	–	2	3	1	1	1	2	–	–	–	–	10
42–43.9	–	–	–	–	1	1	–	1	–	–	–	–	–	–	3
44–45.9	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1
46–47.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
48–49.9	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1
Totals.....	2	9	17	25	42	44	44	25	30	17	10	3	2	2	272

hand, and those in either age, body-weight or stature, on the other hand. The correlation coefficients shown in Table VII confirm this impression.

None of these zero-order coefficients can be regarded as significant, having regard to the probable errors involved. The only reasonable conclusion is that differences between the individuals in this group of normal men in respect of relative cell volume of the blood are not closely correlated with differences between the same individuals in

TABLE V

Correlation of relative cell volume and body-weight. Normal males of Group I

BODY WEIGHT	RELATIVE CELL VOLUME														
	39.5–40.4 per cent	40.5–41.4 per cent	41.5–42.4 per cent	42.5–43.4 per cent	43.5–44.4 per cent	44.5–45.4 per cent	45.5–46.4 per cent	46.5–47.4 per cent	47.5–48.4 per cent	48.5–49.4 per cent	49.5–50.4 per cent	50.5–51.4 per cent	51.5–52.4 per cent	52.5–53.4 per cent	Totals
pounds															
99.5–109.4	—	—	—	1	—	—	1	—	—	—	—	—	—	—	2
109.5–119.4	1	1	1	2	1	—	3	1	1	—	—	1	—	—	12
119.5–129.4	—	—	2	3	1	3	6	2	1	1	—	—	1	—	20
129.5–139.4	—	—	2	5	7	7	8	2	8	2	1	1	—	—	43
139.5–149.4	1	5	2	5	7	13	5	3	6	3	3	—	—	1	54
149.5–159.4	—	1	6	2	11	13	7	4	2	3	1	1	—	—	51
159.5–169.4	—	1	2	5	5	3	3	6	5	4	4	—	—	1	39
169.5–179.4	—	1	1	1	3	2	6	5	4	3	—	—	—	—	26
179.5–189.4	—	—	—	1	6	1	4	2	1	1	—	—	—	—	16
189.5–199.4	—	—	1	—	1	2	1	—	1	—	—	—	—	—	6
199.5–209.4	—	—	—	—	—	—	—	—	1	—	1	—	1	—	3
Totals.....	2	9	17	25	42	44	44	25	30	17	10	3	2	2	272

TABLE VI

Correlation of relative cell volume and stature. Normal males of Group I

STATURE	RELATIVE CELL VOLUME														
	39.5–40.4 per cent	40.5–41.4 per cent	41.5–42.4 per cent	42.5–43.4 per cent	43.5–44.4 per cent	44.5–45.4 per cent	45.5–46.4 per cent	46.5–47.4 per cent	47.5–48.4 per cent	48.5–49.4 per cent	49.5–50.4 per cent	50.5–51.4 per cent	51.5–52.4 per cent	52.5–53.4 per cent	Totals
inches															
59.5–60.4	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
60.5–61.4	—	—	—	—	—	1	2	—	—	—	—	—	—	—	3
61.5–62.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
62.5–63.4	—	—	—	—	—	1	1	—	—	—	—	—	—	—	2
63.5–64.4	—	—	—	—	1	5	3	—	1	2	—	—	—	—	12
64.5–65.4	1	2	—	1	3	1	5	—	1	3	1	1	—	—	19
65.5–66.4	—	—	2	5	3	4	2	—	5	1	4	1	1	1	29
66.5–67.4	—	1	1	2	3	5	8	6	6	6	1	—	1	—	40
67.5–68.4	—	3	5	5	10	8	8	7	2	—	2	—	—	—	50
68.5–69.4	—	—	4	8	5	4	4	3	7	—	—	—	—	—	35
69.5–70.4	—	—	2	2	1	11	4	5	4	3	2	1	—	—	35
70.5–71.4	1	2	2	—	8	2	4	3	1	1	—	—	—	1	25
71.5–72.4	—	1	—	1	6	1	1	1	1	—	—	—	—	—	12
72.5–73.4	—	—	1	—	1	—	2	—	—	1	—	—	—	—	5
73.5–74.4	—	—	—	1	1	—	—	—	2	—	—	—	—	—	4
Totals.....	2	9	17	25	42	44	44	25	30	17	10	3	2	2	272

either age, body-weight, or stature, when each of these characters is considered by itself.

We may next investigate the linearity of regression of relative cell volume on the other variables. The correlation ratios, both crude and corrected for number of arrays, are given in Table VIII (*cf.* Pearson (17)).

For age the crude η is less than $\sqrt{(\kappa - 1)/N}$, the mean value of η in samples from an uncorrelated population, while for body-weight and stature the difference of the values is less than twice $0.67449/\sqrt{N}$,

TABLE VII

Coefficients of correlation between relative cell volume and other variables. Group I

CHARACTERS CORRELATED	VALUE OF COEFFICIENT
Relative cell volume with age: r_{da}	-0.096 ± 0.041
Relative cell volume with body-weight: r_{db}	$+0.114 \pm 0.040$
Relative cell volume with stature: r_{ds}	-0.118 ± 0.040

TABLE VIII

Correlation ratios for relative cell volume

REGRESSION OF RELATIVE CELL VOLUME	CRUDE η	CORRECTED η	$\sqrt{(\kappa-1)/N} = 0.67449/\sqrt{N}$
On age	0.197	Imaginary	0.219 ± 0.041
On body-weight	0.259	0.197	0.192 ± 0.041
On stature	0.294	0.211	0.227 ± 0.041

the probable error of η for samples from an uncorrelated population. On the basis of the correlation ratio, therefore, we cannot assert significant association of relative cell volume with either age, body-weight, or stature, nor significant deviation from linearity of regression.

The values of $\zeta = \eta^2 - r^2$, as calculated from the crude η, are as follows:

On age	0.030 ± 0.014
On body-weight	0.054 ± 0.018
On stature	0.031 ± 0.014

The value for body-weight is three times its probable error, but it should be remembered that this value does not take account of the effect of number of arrays on the value of η.

The means of arrays of relative cell volume on age, body-weight, and stature are given in Table IX.

The equations for the regression of relative cell volume upon the other three variables are, in absolute terms, as follows (a bar over a letter denoting a mean value):

$$\text{Age:} \quad \bar{d} = 46.978 - 0.0453\,a \qquad \text{(i)}$$

$$\text{Body-weight:} \quad \bar{d} = 43.460 + 0.0141\,b \qquad \text{(ii)}$$

$$\text{Stature:} \quad \bar{d} = 53.699 - 0.1190\,c \qquad \text{(iii)}$$

TABLE IX

Means of arrays of relative cell volume on age, body-weight, and stature. Normal males of Group I

TYPE	MEAN OF ARRAY	TYPE	MEAN OF ARRAY	TYPE	MEAN OF ARRAY
years	*per cent*	*pounds*	*per cent*	*inches*	*per cent*
21	45.67	104.5	44.50	60	45.00
23	46.75	114.5	44.75	61	45.67
25	45.97	124.5	45.55	62	
27	45.61	134.5	45.74	63	45.50
29	45.74	144.5	45.30	64	46.08
31	45.45	154.5	45.14	65	45.68
33	45.71	164.5	46.26	66	46.48
35	45.00	174.5	46.08	67	46.50
37	44.60	184.5	45.44	68	44.82
39	44.75	194.5	45.00	69	44.94
41	46.20	204.5	50.00	70	46.23
43	45.33			71	44.92
45	48.00			72	44.50
47				73	45.40
49	46.00			74	45.75

For these equations, and the values of the extreme variates of the characters other than relative cell volume in this sample of material, the following deductions are easily made.

1. A difference in advancing age of 29 years, between the limiting ages of 20 and 49, has associated with it a decrease of relative cell volume of 1.314 per cent (= 46.072 at age 20 − 44.758 at age 49).

2. A difference in increasing body-weight of 100 pounds, between the limiting weights of 105 pounds and 205 pounds, has associated with it an increase of relative cell volume of 1.410 per cent.

3. A difference in increasing stature of 14 inches, between the limit-

ing heights in the sample of 60 and 74 inches, has associated with it a decrease of relative cell volume of 1.666 per cent.

It is apparent again from these values that the element of the internal environment of normal man represented by relative cell volume of the blood is a comparatively stable and constant thing. Wide differences between individuals in respect of age (adult), body-weight and stature have associated with them only relatively small differences in this characteristic of the blood.

The other zero-order correlation coefficients, necessary for the computation of the net correlations, are given in Table X.

TABLE X

Zero-order correlations between age, body-weight, and stature. Group I

CHARACTERS CORRELATED	VALUE OF COEFFICIENTS
Age with body-weight: r_{ab}	-0.052 ± 0.041
Age with stature: r_{as}	-0.173 ± 0.040
Body-weight with stature: r_{bs}	$+0.427 \pm 0.033$

By a well-known technique (*cf.* Pearl (18)) the following net or partial correlation coefficients are easily derived from the data already presented:

Net correlation between relative cell volume and age, with body-weight and stature constant $r_{da.bs} = -0.125 \pm 0.040$

Net correlation between relative cell volume and body-weight, with age and stature constant $r_{db.as} = +0.187 \pm 0.040$

Net correlation between relative cell volume and stature, with age and body-weight constant $r_{ds.ab} = -0.204 \pm 0.039$

While all of these net correlations are more than three times their probable errors, all are absolutely of small magnitude. This merely expresses again the relative conservatism and stability, inter-individually, of the cell volume of the blood. Such associational tendencies in this character as are indicated by these correlations are in the sense that relative cell volume tends to be smaller in older men than in younger, within the period from 20 to 49 years, when body-weight and stature are held constant. It also tends to be smaller in tall men than in short, when age and body-weight are held constant. The relative cell volume of the blood tends to be higher in the heavier men

out of a group of normal men of the same age and stature, than in the lighter.

Both gross and net correlations are higher for the association of relative cell volume with body-weight than for its association with age. The highest correlation, both gross and net, which relative cell volume exhibits within the present material, is with stature. Remembering that this is still true when the effects of age and body-weight have been eliminated, the suggestion comes to one's mind that relative cell volume of the blood may be characteristically different in individuals of different constitutional types. The point seems worth special investigation. It cannot, of course, be finally determined from the present material.

Let us pursue this matter a little further, however. In the present group of 272 men the lowest relative cell volume found in any individual was 40 per cent, and the highest was 53 per cent. Each of these extreme values was found in two men. It therefore follows that a relative cell volume of anything between 40 per cent and 53 per cent is compatible with physiological normality, health and well-being, because all these men were physiologically normal and healthy. But it is shown above from regression equations (i) to (iii) that the extreme difference in relative cell volume associated with a difference of 29 years in age amounts to only 1.314 per cent. This difference is only one-tenth of that which is compatible with physiological normality and health. Similarly the differences in the general biological organization of the body which are implicate in differences of 100 pounds in body-weight and 14 inches in stature have associated with them differences in relative cell volume amounting again roughly to only a little more than one-tenth of the physiologically permissible variation in this characteristic of the blood.

Henderson's (2) nomogram shows that the alteration in relative cell volume associated with the extreme limits of physiological regulatory changes in the blood amounts roughly to only about one-twentieth of the range of variation in this character found in the present study to be compatible with physiological normality and health.

The complete meaning of these facts, from the viewpoint of theoretical biology, is obscure, but we think a conservative provisional conclusion is that relative cell volume is invariant, to all but a slight and

comparatively insignificant degree, relative to two important biological reference-frames, namely: (1) Age-external-environment, within the limits imposed by the maintenance of physiological normality. (2) Stature-body-weight, and the constitutional organization implied by them.

This in turn implies that relative cell volume is probably itself an independent element in the total organization pattern, or at least that whatever important association it may have is with quite other elements in the total pattern of the organism than those directly implicate in 1 and 2 above. The natural suggestion is that relative cell volume is closely associated with the other elements which determine the internal

TABLE XI

Mean values of relative cell volume predicted from net regression equation. Normal males

AGE	MEAN RELATIVE CELL VOLUME OF BLOOD	
	a. For groups of men having the mean body-weights and statures associated with the stated age	b. For groups of men each having the mean body-weight and stature of the whole present sample
	per cent	*per cent*
20	46.08	46.22
25	45.85	45.93
30	45.62	45.63
35	45.40	45.34
40	45.17	45.04
45	44.95	44.75
50	44.72	44.46

environment. But in view of what Henderson's nomogram shows, and remembering that the biochemical-physiological behavior of the blood is easily the most important element in the determination and regulation of the internal environment, such a conclusion must not be too hastily drawn.

The net regression equation, connecting relative cell volume with the other three variables is

$$\bar{d} = 59.13 - 0.0588\,a + 0.0253\,b - 0.2285\,c \qquad \text{(iv)}$$

From this equation the values of Table XI are computed.

It seems clear from Table XI, as well as from all the evidence that has preceded, that relative cell volume tends to have somewhat lower

values in persons of higher ages, at least within the age limits of the present sample of material. It is highly desirable to have data on relative cell volume in persons outside the age range of the present sample, and especially in very old people. Within the age range here under discussion the regression of relative cell volume on age follows about the course which has been observed for a variety of characters, such as brain weight (19), stature (19), spleen weight (20), etc. There is a linear decline in its value with advancing age, between the early twenties and fifty years of age. The rate of this decline is small, but it is definite.

There is, of course, always a tendency to conclude that such regressions as these mark senescent changes—that, for example, in the present instance, the relative cell volume is lower in the older men in

TABLE XII

Observed and calculated (equation iv) relative cell volume for 4 normal men

INDEX NUMBER	OBSERVED			RELATIVE CELL VOLUME	
	Age	Body-weight	Stature	Observed	Calculated by equation (iv)
6	22	105	61	46	46.6
30	26	180	69	44	46.4
93	26	145	68	43	45.7
115	28	135	69	48	45.1

the group, on the average, than it is in the younger, *because* the relative cell volume of each individual decreases slightly as he grows older.

But such a conclusion is unwarranted from the *kind* of evidence here presented. It may be true, one may even say perhaps that it probably is true, but the only way to *prove*, or really to find out, that it is, is to measure the relative cell volume of the *same individual* man at successive ages, and do this with enough different men to be sure that a normal result is obtained. All that the correlation method tells us, or ever can tell, is that slightly lower average relative cell volumes are found in groups of older men than are found in groups of younger men. The method can tell us nothing directly about how this came to be so.

Equation (iv) may be used, of course, to predict the probable relative cell volume of normal men having any given age, body-weight,

and stature. In so using the equation it must always be remembered that it gives $\bar{d}$, that is the *mean* of an array. It will predict well the relative cell volume of an individual only if that individual happens to lie near the mean of his array. How it works in practice is shown in Table XII, in which 4 actual individuals are taken at random from Group I, and equation (iv) used to predict their probable relative cell volume.

Except in the case of the first of these random individuals (No. 6) the predictions are very poor. This is precisely what any competent biometrician would expect them to be. The correlations upon which equation (iv) is based are nearly all low, some of them not significantly different from zero. Hence the multiple regression equation cannot be expected to predict individual values with any marked degree of dependability. We have thought it worth while to present the concrete example of Table XII to show the unreliability of such an equation as (iv) for individual predictions, because we think that similar multiple regression equations are being used in certain physiological and medical fields at the present time in a quite uncritical way. Non-mathematical persons are most easily hoodwinked by a brave display of equations. Our equation (iv), and all similar multiple regression equations, can be legitimately used only to predict the mean value of an array, and even there, in the present case, the theoretical standard deviation of the predicted array, in relative cell volume, is 2.40 per cent. But Table I shows that the standard deviation of the entire normal sample (Group I), in relative cell volume, is 2.47 per cent. So then by correcting for age, body-weight, and stature we reduce the variability in relative cell volume by only 0.07 of a unit.

IV

In the preceding sections we have discussed only the normal males. There are also available in Dr. Schlomovitz's data two other groups, as follows:

Group II consists of 449 men in hospital because of active tuberculosis of the lungs.

Group III consists of 50 men, about to be discharged as arrested or healed cases of pulmonary tuberculosis.

The same kinds of data are available for these two groups as were

for Group I. We shall turn now to a biometric examination of relative cell volume and correlated characters in these tuberculous Groups II and III.

The general characteristics of the distribution of variation in relative cell volume of the blood in Groups II and III may be first examined graphically and compared with the findings for normal males. Figure 3 is plotted on the same basic plan as that used for Figure 2. The abscissae give percentage values of relative cell volume for Group I (normal males), II, and III, the mean for each group being taken as

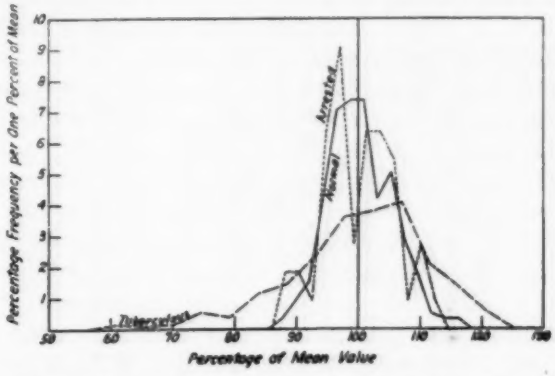

FIG. 3. FREQUENCY POLYGONS FOR VARIATION IN RELATIVE CELL VOLUME IN GROUP I (NORMAL MALES), SOLID LINE; GROUP II (ACTIVELY TUBERCULOUS MALES), DASH LINE; GROUP III (ARRESTED TUBERCULOUS MALES) DOT LINE

For explanation of method of plotting see text

100 per cent. The ordinates are relative (percentage) frequencies per one per cent of the mean for each group. This procedure brings all these variation polygons to the same area.

It is at once apparent from inspection of Figure 3 that relative cell volume varies much more in the actively tuberculous group than in the normal. The arrested Group III shows about the same degree of scatter of the individuals as does the normal Group I, making allowance for the small size of Group III and the consequent irregularities in the distribution.

TABLE XIII

Variation constants for Groups II and III

CHARACTER	MEAN	MEDIAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
		Group II. Active tuberculosis		
a. Age	31.49 ± 0.14 years	31.26 ± 0.18 years	4.26 ± 0.10 years	13.52 ± 0.31 per cent
b. Body-weight	137.46 ± 0.60 pounds	136.43 ± 0.75 pounds	18.82 ± 0.42 pounds	13.69 ± 0.31
c. Stature	68.49 ± 0.08 inches	68.30 ± 0.11 inches	2.65 ± 0.06 inches	3.86 ± 0.09
d. Relative cell volume	43.52 ± 0.15 per cent	44.05 ± 0.19 per cent	4.84 ± 0.11 per cent	11.13 ± 0.25
		Group III. Arrested tuberculosis		
a. Age	32.44 ± 0.34 years	32.36 ± 0.43 years	3.56 ± 0.24 years	10.96 ± 0.75 per cent
b. Body-weight	151.30 ± 1.84 pounds	146.83 ± 2.30 pounds	19.33 ± 1.30 pounds	12.78 ± 0.88
c. Stature	69.12 ± 0.25 inches	69.50 ± 0.31 inches	2.61 ± 0.18 inches	3.77 ± 0.25
d. Relative cell volume	45.38 ± 0.25 per cent	45.50 ± 0.31 per cent	2.60 ± 0.18 per cent	5.73 ± 0.39

The means, medians, standard deviations, and coefficients of variation for Groups II and III are given in Table XIII, together with their probable errors.

From Table XIII the following points are to be noted:

1. Just as in the normal males of Group I the variation in relative cell volume is quite symmetrically grouped about the mean value in the tuberculous samples. There is more of a tendency towards skewness in variation in the actively tuberculous group (II) than in either of the others, but even in Group II the difference between mean and median is only a little more than twice its probable error, and cannot be regarded as statistically significant.

2. As would be expected, the mean body-weight of the individuals in the actively tuberculous group (II) is significantly lower than that of the individuals in either of the other two groups. In mean age Group II is 0.90 ± 0.25 years older than Group I, while Group III is 0.95 ± 0.37 years older than Group II, and 1.85 ± 0.40 years older than Group I. In mean stature Group II (actively tuberculous) is 0.36 ± 0.13 inches taller than Group I (normal). Group III (arrested tuberculous) is 0.63 ± 0.26 inches taller than Group II, and 0.99 ± 0.27 inches taller than Group I.

3. The mean relative cell volume is distinctly *lower* in the actively tuberculous group (II) than it is in either the normals (I) or the arrested tuberculous (III). The amounts of these differences are as follows:

Difference in mean relative cell volume between Groups I and II	$= -2.07 \pm 0.18$
Difference in mean relative cell volume between Groups III and II	$= -1.86 \pm 0.31$

These differences, as they stand, are certainly significant statistically. But before discussing the possible meaning of this result it is necessary to wait until we have in hand the data necessary to correct for whatever differences there are between the several groups in respect of the other characters correlated with relative cell volume.

Schlomovitz (1) and his coworkers have concluded from their study that:

"The chronic ulcerative type of pulmonary tuberculosis is quite commonly accompanied by, or rather, responsible for a disintegration of certain blood ele-

ments. The significance of the factor of relative corpuscular volume has not been emphasized heretofore.

"The relative blood cell volume bears a constant relation to the patient's condition. A high value is found in those who are progressing favorably, and a low value in those who are rapidly deteriorating physically."

4. In respect of all characters discussed except age, Group II (actively tuberculous) is more variable, as measured by the coefficient of variability, than either of the other two groups. In age the normal group (I) is relatively the most variable. In respect of stature and body-weight the extent to which Group II is more variable than Groups I or III is not significant. But in relative cell volume the difference in variability is large and significant. The differences in the coefficients of variability for this character are as follows:

$$\begin{aligned} \text{Group II} - \text{Group I} &= +5.71 \pm 0.30 \\ \text{Group II} - \text{Group III} &= +5.40 \pm 0.46 \\ \text{Group III} - \text{Group I} &= +0.21 \pm 0.42 \end{aligned}$$

Roughly the actively tuberculous group (II) is about twice as variable in respect of relative cell volume as is the normal group (I). The arrested group (III) exhibits a degree of variation in this character which is identical, within the probable error, with that shown by the normal group.

Evidently the existence in the individual of active pulmonary tuberculosis has associated with it a marked alteration from the normal of that particular element of the internal environment which is measured by the relative cell volume of the blood. Not only is the type significantly different, but the inter-individual stability or constancy, which was found to be so striking in the normal group, has disappeared.

V

The zero-order correlation tables, for the correlation of relative cell volume with age, body-weight, and stature in the actively tuberculous group, are presented as Tables XIV to XVI.

The correlation coefficients derived from these tables, and also the corresponding coefficients for Group III (arrested tuberculosis), are shown in Table XVII.

In the actively tuberculous group (II) the only character with which relative cell volume is sensibly correlated is body-weight. This correlation is positive in size and is 10 times its probable error. It is in accord with what might be expected on general physiological and pathological grounds. The body-weight of a person with active pulmonary tuberculosis is a rather reliable index of the progress of his malady. And apparently relative cell volume also is. Hence it

TABLE XIV

Correlation of relative cell volume with age among actively tuberculous males. Group II

AGE	RELATIVE CELL VOLUME														
	25.5–27.4 per cent	27.5–29.4 per cent	29.5–31.4 per cent	31.5–33.4 per cent	33.5–35.4 per cent	35.5–37.4 per cent	37.5–39.4 per cent	39.5–41.4 per cent	41.5–43.4 per cent	43.5–45.4 per cent	45.5–47.4 per cent	47.5–49.4 per cent	49.5–51.4 per cent	51.5–53.4 per cent	Totals
years															
20.0–21.9	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
22.0–23.9	—	—	—	—	—	—	1	—	1	1	2	1	1	1	8
24.0–25.9	—	—	—	—	—	1	5	4	6	7	6	5	1	1	36
26.0–27.9	1	—	1	1	1	3	3	4	8	12	4	5	4	—	47
28.0–29.9	1	1	1	3	2	6	8	7	13	11	21	7	4	1	86
30.0–31.9	—	—	1	—	1	7	5	7	9	16	11	9	7	1	74
32.0–33.9	—	1	—	4	1	1	2	15	13	11	13	4	7	3	75
34.0–35.9	1	—	—	1	2	4	3	5	7	9	13	5	3	1	54
36.0–37.9	—	—	—	2	1	1	2	4	8	5	7	4	—	1	35
38.0–39.9	—	—	—	—	—	1	1	1	9	5	3	1	1	3	25
40.0–41.9	—	—	—	—	—	—	—	1	—	1	2	1	—	—	5
42.0–43.9	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
44.0–45.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
46.0–47.9	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
48.0–49.9	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
Totals.....	3	2	3	11	8	24	30	48	74	78	84	43	29	12	449

would be expected that these two variables would be positively correlated.

The arrested group (III) shows substantially the same system of zero-order correlations as does the normal group (I) discussed earlier. The only difference is that the body-weight correlation is negative instead of positive in sign. But since in neither group can the coefficient be looked upon as statistically different

from zero, having regard to the probable errors, the difference in sign in the two cases probably has no biological meaning.

The regressions for both Group II and Group III are linear, within the limits of error of sampling. The equations for the regression of relative cell volume on the other three variables are as follows:

Group II (active tuberculosis)

$$\bar{d} = 43.129 + 0.0123\,a \quad \text{(v)}$$
$$\bar{d} = 33.180 + 0.0752\,b \quad \text{(vi)}$$
$$\bar{d} = 42.262 + 0.0183\,c \quad \text{(vii)}$$

TABLE XV

Correlation of relative cell volume with body-weight among actively tuberculous males. Group II

BODY-WEIGHT	RELATIVE CELL VOLUME														
	25.5–27.4 per cent	27.5–29.4 per cent	29.5–31.4 per cent	31.5–33.4 per cent	33.5–35.4 per cent	35.5–37.4 per cent	37.5–39.4 per cent	39.5–41.4 per cent	41.5–43.4 per cent	43.5–45.4 per cent	45.5–47.4 per cent	47.5–49.4 per cent	49.5–51.4 per cent	51.5–53.4 per cent	Totals
pounds															
89.5– 99.4	—	1	—	—	1	—	3	1	—	—	—	—	—	—	6
99.5–109.4	1	1	1	1	—	3	4	2	4	3	—	—	—	1	21
109.5–119.4	—	—	—	3	—	5	4	7	8	4	3	3	1	2	40
119.5–129.4	2	—	—	1	2	6	8	10	10	16	15	7	5	2	84
129.5–139.4	—	—	2	3	2	7	6	9	20	19	15	14	6	3	106
139.5–149.4	—	—	—	—	1	—	3	13	17	22	22	10	6	1	95
149.5–159.4	—	—	—	2	1	—	2	3	4	9	17	3	4	1	46
159.5–169.4	—	—	—	1	1	1	—	2	8	3	5	4	1	—	26
169.5–179.4	—	—	—	—	—	1	—	1	—	—	4	1	4	—	11
179.5–189.4	—	—	—	—	—	1	—	—	2	2	3	1	1	1	11
189.5–199.4	—	—	—	—	—	—	—	—	—	—	—	—	1	1	2
199.5–209.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
209.5–219.4	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
Totals.....	3	2	3	11	8	24	30	48	74	78	84	43	29	12	449

Group III (arrested tuberculosis)

$$\bar{d} = 47.297 - 0.0591\,a \quad \text{(viii)}$$
$$\bar{d} = 47.235 - 0.0123\,b \quad \text{(ix)}$$
$$\bar{d} = 62.750 - 0.2513\,c \quad \text{(x)}$$

The additional zero-order coefficients for Groups II and III, necessary for the computation of net coefficients, are presented in Table XVIII.

These coefficients are of the same general order of magnitude as the corresponding ones for the normal group (I). The net or partial

TABLE XVI

Correlation of relative cell volume with stature among actively tuberculous males. Group II

STATURE	RELATIVE CELL VOLUME														
	25.5–27.4 per cent	27.5–29.4 per cent	29.5–31.4 per cent	31.5–33.4 per cent	33.5–35.4 per cent	35.5–37.4 per cent	37.5–39.4 per cent	39.5–41.4 per cent	41.5–43.4 per cent	43.5–45.4 per cent	45.5–47.4 per cent	47.5–49.4 per cent	49.5–51.4 per cent	51.5–53.4 per cent	Totals
inches															
60.5–61.4	—	—	—	—	—	—	—	—	—	1	1	—	—	—	2
61.5–62.4	—	—	—	—	—	—	—	—	2	—	—	—	1	—	3
62.5–63.4	—	—	—	—	—	1	—	1	2	1	1	2	—	1	9
63.5–64.4	—	—	—	1	1	2	2	1	2	1	2	1	1	1	15
64.5–65.4	—	—	1	—	—	4	—	2	4	5	4	2	4	—	26
65.5–66.4	—	—	—	1	2	2	7	4	7	6	7	6	2	1	45
66.5–67.4	1	1	—	—	—	2	4	10	11	10	17	7	1	1	65
67.5–68.4	—	—	2	4	1	3	5	8	12	11	7	13	4	4	74
68.5–69.4	—	1	—	2	—	3	1	6	11	7	9	2	3	—	45
69.5–70.4	—	—	—	1	2	4	4	6	5	12	11	5	5	—	55
70.5–71.4	—	—	—	1	2	1	4	6	5	12	10	3	2	2	48
71.5–72.4	1	—	—	—	—	1	1	3	7	7	10	1	4	2	37
72.5–73.4	1	—	—	—	—	1	1	1	4	3	2	—	—	—	13
73.5–74.4	—	—	—	1	—	—	1	—	1	2	2	1	1	—	9
74.5–75.4	—	—	—	—	—	—	—	—	—	—	1	—	1	—	2
75.5–76.4	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
Totals.....	3	2	3	11	8	24	30	48	74	78	84	43	29	12	449

TABLE XVII

Coefficients of correlation between relative cell volume and other variables. Groups II and III

CHARACTERS CORRELATED	GROUP II (ACTIVE TUBERCULOSIS)	GROUP III (ARRESTED TUBERCULOSIS)
Relative cell volume with age: r_{da}.....	$+0.011 \pm 0.032$	-0.081 ± 0.095
Relative cell volume with body weight: r_{db}...	$+0.292 \pm 0.029$	-0.091 ± 0.095
Relative cell volume with stature: r_{ds}.....	$+0.010 \pm 0.032$	-0.252 ± 0.089

correlation coefficients for the tuberculous groups (II and III) are given in Table XIX.

From these values, in connection with the data for the normals already presented, it appears that:

1. The signs of the several net correlations are the same in all three groups. This suggests that, small as the individual coefficients for the most part are, there is perhaps reflected here a definite correlational nexus or pattern between the four characters considered.

2. The net correlations for Group III are not significantly different

TABLE XVIII

Zero-order correlations between age, body-weight, and stature. Groups II and III

CHARACTERS CORRELATED	GROUP II (ACTIVE TUBERCULOSIS)	GROUP III (ARRESTED TUBERCULOSIS)
Age with body-weight: r_{ab}	-0.037 ± 0.032	-0.073 ± 0.095
Age with stature: r_{as}	-0.157 ± 0.031	$+0.055 \pm 0.095$
Body-weight with stature: r_{bs}	$+0.515 \pm 0.023$	$+0.480 \pm 0.073$

TABLE XIX

Net or partial correlation coefficients for relative cell volume in the tuberculous (Groups II and III)

CHARACTERS CORRELATED	CHARACTERS HELD CONSTANT	SYMBOL	GROUP II (ACTIVE TUBERCULOSIS)	GROUP III (ARRESTED TUBERCULOSIS)
Relative cell volume and age	Body-weight and stature	$r_{da \cdot bs}$	-0.005 ± 0.032	-0.066 ± 0.095
Relative cell volume and body-weight	Age and stature	$r_{db \cdot as}$	$+0.335 \pm 0.028$	$+0.027 \pm 0.095$
Relative cell volume and stature	Age and body-weight	$r_{ds \cdot ab}$	-0.170 ± 0.031	-0.231 ± 0.090

from those for Group I. The net correlation between relative cell volume and body-weight is probably significantly higher in Group II than it is in Group I.

The multiple regression equations for Groups II and III are:

Group II (active tuberculosis): $\bar{d} = 53.96 - 0.0054\,a + 0.1006\,b - 0.3519\,c$ (xi)

Group III (arrested tuberculosis): $\bar{d} = 64.42 - 0.0469\,a + 0.0041\,b - 0.2624\,c$ (xii)

We come now to the consideration of an important point. To what extent is the lowered value of the mean relative cell volume in

the actively tuberculous group (II) (shown in Table XIII) as compared with the normals, to be accounted for by differences in age, body-weight and stature between the two groups? This question may be approached in either of two ways. We may, on the one hand, calculate from equation (iv) what would be the probable mean relative cell volume of an array of normal men having the same mean age, body-weight, and stature as do the men of the actively tuberculous Group II; or, on the other hand, we may calculate from equation (xi) the probable mean relative cell volume of an array of actively tuberculous men who had the same mean age, body-weight and stature, as do the normal men of Group I. Applying these methods we got the following results:

A tuberculous male array with the same age, body-weight, and stature as the means of these characters in the normal Group I would have, from multiple regression equation (xi), a mean relative cell volume of 45.07 per cent, as compared with a mean relative cell volume of 45.59 $\pm$ 0.10 per cent actually observed in the normal Group I. Reversing the procedure, an array of normal males with the mean age, body-weight, and stature of the actively tuberculous Group II would have, from equation (iv), a mean relative cell volume of 45.10 per cent, as compared with 43.52 $\pm$ 0.15 per cent observed in the tuberculous.

It is thus seen that if reliance were put exclusively upon equation (xi) one would conclude that nearly all of the observed difference between normal and tuberculous persons in mean relative cell volume is accounted for by age, body-weight, and stature differences between the two groups. In other words, the mean relative cell volume of a group of tuberculous persons can apparently be adjusted nearly to normal simply by manipulation of their age, body-weight, and stature to coincide with those of the normals. But it seems to be impossible to adjust the mean relative cell volume of normal persons to that exhibited by the actively tuberculous by any such process. And in the first case the process does not give quite an accurate result. The predicted value from equation (xi) is, after all, a half unit lower than that actually observed in normal males.

Taking all the evidence into account it seems probable that the presence of active pulmonary tuberculosis has as one of its associated

phenomena a lowering of the relative cell volume of the blood, just as was concluded by Schlomovitz and his coworkers (1). There would thus seem to be added another bit of evidence to the already large amount existing, that this disease fundamentally alters the structural-functional pattern of the organism.

Using the same method as above we get the following results regarding Group III (arrested tuberculosis).

An array of males having arrested tuberculosis with the mean age, body-weight, and stature of the normal group, would have, by equation (xii), a mean relative cell volume of 45.74 per cent as compared with the observed 45.59 $\pm$ 0.10 per cent. A normal group with the mean age, body-weight, and stature of the arrested tuberculous Group III would have, by equation (iv) a mean relative cell volume of 45.25 per cent, as compared with the observed 45.38 $\pm$ 0.25 per cent in Group III. There is therefore no significant difference in relative cell volume between the normal and the arrested tuberculous.

SUMMARY

This paper presents the results of a biometric analysis of inter-individual variation in relative cell volume of the blood and certain correlated variables in 272 normal males, ranging in age from 20 to 49 years; 449 actively tuberculous males, ranging in age from 21 to 48 years; and 50 men about to be discharged from hospital as arrested or healed cases of tuberculosis, ranging in age from 25 to 40 years. It is shown that:

1. In normal males the distribution of variation in relative cell volume about the mean value of 45.59 $\pm$ 0.10 per cent is substantially symmetrical, and relatively small in amount, as compared with most other physiological characters of the organism. This low variability is held to be related to the fact that relative cell volume of the blood is one index or attribute of the internal environment of the organism, the marked stability of which has been discussed by various physiologists.

2. Individual differences or variations in relative cell volume, in the group of normal males, are not highly correlated with differences in either age, body-weight, or stature in the same individuals, either in

gross or net. The regressions of these variables upon each other, within the range of the present sample, are all linear. The regression of relative cell volume upon each of the other variables considered is small in amount, much smaller than the range of difference in this character compatible with physiological normality and health. Relative cell volume is most highly correlated, both in gross and net, with stature, the sign of correlation being negative; next with body-weight, the sign being positive; and least correlated with age, the sign being negative.

3. Multiple regression equations are presented for the estimation of the probable mean relative cell volume associated with any given age, body-weight, and stature, for each of the three groups discussed. The significance and limitations of these equations are pointed out.

4. A novel method of graphically exhibiting comparative variability is described and illustrated.

5. The mean relative cell volume of the blood is markedly lower in the actively tuberculous males than it is in the normal males. This difference is not entirely to be accounted for by differences in age, body-weight, and stature existing between the groups. It seems probable that the presence of active pulmonary tuberculosis has as one of its associated phenomena a lowering of the relative cell volume of the blood.

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CONTRIBUTION TO THE STUDY OF TOXIC ABSORPTION FROM THE INTESTINAL TRACT IN EXPERIMENTAL HIGH OBSTRUCTION

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The contents of a closed loop in the upper part of the intestinal tract of a dog, when injected intravenously into another animal, produce symptoms quite similar to those observed in high intestinal obstruction. The animal into which the contents are injected usually dies even when small amounts of the closed loop material are employed. The implication that *intestinal toxin* is the lethal factor in intestinal obstruction is therefore deduced from this experiment and this explanation of the cause of death is generally accepted, although a few workers who have studied the condition experimentally disagree with this theory. Recent work (1) has confirmed the earlier findings as regards intestinal obstruction.

Experimental work would seem to justify one in regarding the intestinal wall as a membrane conforming in some respects to the physical laws of diffusion and osmosis (2). Other properties of the membrane, however, especially those related to cellular physiology, undoubtedly play an important rôle in the behavior of the intestinal wall. In considering absorption phenomena as purely physiochemical in nature, a number of factors have to be considered. Molecular concentration of fluids, surface tension, polarization of the membrane, electro-motor activity, imbibition, etc., all may figure in the problem of absorption and must be kept in mind. Recent work (3) has shown that a collodion filter, which is impermeable to hemoglobin, may be rendered permeable by the addition of sodium oleinate or sodium glycocholate. It seemed worth while, therefore, to investigate the effect of such substances upon the absorption of toxin by the intestinal wall, although Buglia and later Török (5), experimenting with normal

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TABLE I

Experiments on the action of soap or bile, with toxin from obstructed gut, suspended in living loops of intestine, and the surrounding Ringer's solution injected into test animals

Toxin from Dog A without soap	Living loop from Dog 1	Test animal lived after injection
Toxin from Dog A with soap	Living loop from Dog 1	Test animal lived
Toxin from Dog A without soap	Loop from Dog 2	Test animal lived
Toxin from Dog A with soap	Loop from Dog 2	Test animal lived
Toxin from Dog A without soap	Loop from Dog 3	Test animal lived
Toxin from Dog A with soap	Loop from Dog 3	Test animal died after 3 days with distemper
Toxin from Dog B without soap	Loop from Dog 4	Test animal died after 3 days (no typical lesions)
Toxin from Dog B with soap	Loop from Dog 4	Test animal died after 5 days (no typical lesions)
Toxin from Dog B without soap	Loop from Dog 5	Test animal lived
Toxin from Dog B with soap	Loop from Dog 5	Test animal lived
Toxin from Dog B without soap	Loop from Dog 6	Test animal died after 20 hours (distemper; no typical lesions)
Toxin from Dog B with soap	Loop from Dog 6	Test animal lived
Toxin from Dog C without soap	Loop from Dog 7	Test animal lived
Toxin from Dog C with soap	Loop from Dog 7	Test animal lived
Toxin from Dog C without soap	Loop from Dog 8	Test animal died after 6 hours with typical lesions of loop toxin poisoning
Toxin from Dog C with soap	Loop from Dog 8	Test animal lived

Toxin from Dog C without soap	Loop from Dog 9	Test animal lived
Toxin from Dog C with soap	Loop from Dog 9	Test animal lived
Toxin from Dog D without bile	Loop from Dog 10	Test animal lived
Toxin from Dog D with bile	Loop from Dog 10	Test animal lived
Toxin from Dog E without bile	Loop from Dog 11	Test animal lived
Toxin from Dog E with bile	Loop from Dog 11	Test animal lived
Toxin from Dog E without bile	Loop from Dog 12	Test animal (in poor condition) dead after 2 days; no typical lesion
Toxin from Dog E with bile	Loop from Dog 12	Test animal lived
Toxin from Dog E without bile	Loop from Dog 13	Test animal dead in 12 hours without typical lesions
Toxin from Dog E with bile	Loop from Dog 13	Test animal lived
Toxin from Dog F without bile	Loop from Dog 14	Test animal lived
Toxin from Dog F with bile	Loop from Dog 14	Test animal lived
Toxin from Dog F without bile	Loop from Dog 15	Test animal lived
Toxin from Dog F with bile	Loop from Dog 15	Test animal lived

small intestine, found that bile and bile salts did not influence the rate of absorption.

In these experiments four dogs were used for each typical procedure. In the first dog a closed duodenojejunal loop (15 cm.) was made either by double ligation of the bowel or by section at two points and inversion of the ends of the loop. This dog was allowed to die, the contents of the loop being removed and preserved in the ice-box until required. This furnished the material with which the study of absorption was made. Its toxicity was determined by injecting it intravenously into small dogs and only loop contents which caused death in a few hours with the typical lesions of congestion and hemorrhage in the duodenum were used for further experiments. The second dog used in these studies provided two normal living loops of bowel for use as absorbing membranes. The duodenojejunal loop was quickly excised from this dog, divided, and both parts were suspended in Ringer's solution in a water-bath, and kept alive in this manner for one hour during which the experiment lasted. The viability of the muscle was demonstrated by the fact that it continued to contract during the time of the experiment and it was assumed that the epithelium was also living. The toxin was put into the lumen of these suspended loops, the ends of which were tied. The test for penetration of the toxic material within the loops through the wall of the bowel was made by injecting the surrounding Ringer's solution intravenously into a third and a fourth dog. To recapitulate, Dog 1 produced the toxin, Dog 2 provided the loops of normal bowel to be tested and Dogs 3 and 4 were the test animals into which the Ringer's solution was injected for its toxic effects.

Nine experiments were performed to determine the influence of soap upon the passage of the toxic substances through the intestinal wall; sufficient castile soap was added to the loop contents to make a one per cent solution. Six of these experiments seemed to demonstrate conclusively that under the conditions mentioned soap influenced the passage of such toxin only to a very slight extent. However, some of the poisonous material must have passed through the bowel wall, for all the dogs showed more or less toxic symptoms, such as vomiting, diarrhea and dyspnea. In two experiments the results were not entirely satisfactory, for both the controls died rather late and the symp-

toms and pathological findings were not typical of obstruction toxin. Neither of these animals presented the duodenojejunal congestion and sub-mucous hemorrhage. Both of them had distemper and were in poorer condition than they seemed to be. Experiment 8 demands consideration; the control animal was injected with Ringer's solution, surrounding a loop which contained toxin but no soap. This animal died in less than six hours with typical symptoms and definite pathological changes in the duodenum. Another dog which was injected with the same toxin suspended in the loop, plus soap solution, recovered promptly. While this one experiment would not justify the assumption that soap has an inhibitory action on the passage of toxin through the bowel wall, the series as a whole seems to confirm the findings of others, that soap in no way favors the absorption of such toxins.

The same conclusions were reached concerning the action of bile. Bile does not favor the diffusion of toxic products at least during the period of one hour. One experiment out of the six performed is doubtful, as the control died after two days. This animal, however, was in poor condition and at autopsy no characteristic lesions in the duodenum were found. Another experiment suggested that bile may have an inhibitory action on the passage of toxin through the intestinal wall. This is in accord with the conclusion arrived at by Mellanby (4) who showed that bile in the intestinal canal delayed very markedly the absorption of histamin.

SUMMARY AND CONCLUSIONS

1. In our experiments an attempt was made to influence the passage of toxins obtained from an obstructed bowel through the wall of an isolated loop of intestine.

2. It was found that under the conditions of these experiments a one per cent solution of soap did not influence appreciably the passage of the toxin through the intestinal wall.

3. A similar experiment, made with bile added to the intestinal toxin in the proportion of one to ten, leads to the same conclusion, namely, that diffusion is not materially influenced by the presence of bile.

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A SUPRA-VITAL STUDY OF THE CELLS IN THE LYMPH STREAM OF THE RABBIT[1]

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INTRODUCTION

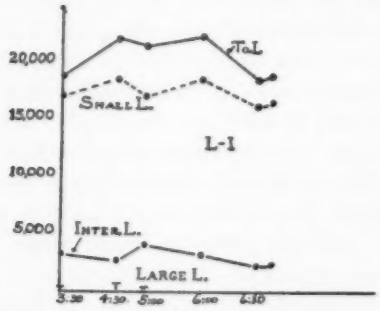
An examination of the literature reveals that comparatively few studies have been made of the cells that circulate in the lymph. Practically all such studies as have been published have been based upon data derived from fixed preparations. The use of such preparations, although for years the sole basis of hematologic study, has recently been more and more supplemented, and to great advantage, by the method of supra-vital staining, described by Sabin (8). The more one uses the method, the more does one feel that it makes possible much clearer differentiations of cell-types than can be attained by the use of fixed preparations. The increased interest which has recently begun to be aroused in the rôle of the lymphocyte in the animal economy, and more specifically, the evidence of the importance of such a rôle, which seemed to come out from the recent study by Cunningham, Sabin, and their co-workers (2) of tuberculosis in the rabbit, and also the recent work of Murphy (7), suggested the present study. The rabbit was used because, although the technical difficulties were greater than might be the case with some other animals, it was desired to have data directly comparable with those of the tuberculosis experiments mentioned. The apparent alternation of small and intermediate lymphocytes in the human blood-stream, as found by Sabin and her co-workers (9), also raised the question as to the relative numbers of these forms in the lymph-stream.

LITERATURE

Davis and Carlson (3), using the dog, found that the lymph contained on an average about 30,000 cells per cubic millimeter, of which

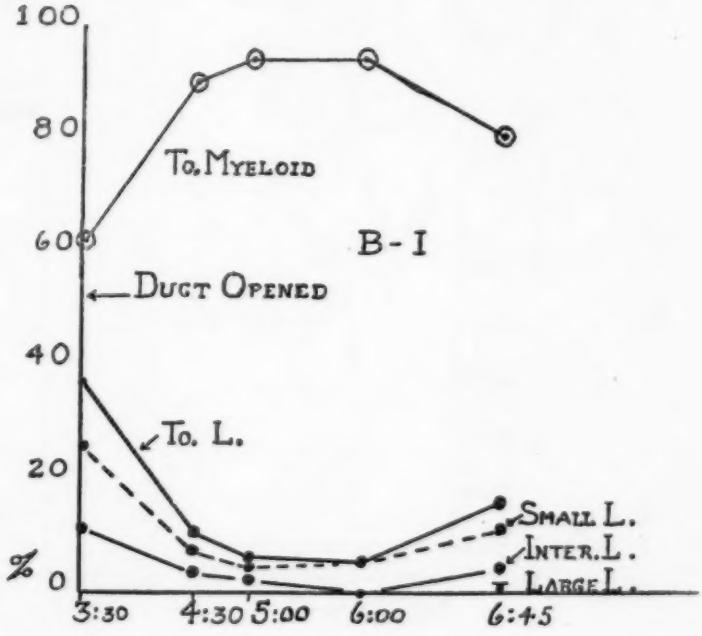
[1] Done under a grant from the Henry Strong Denison Medical Foundation.

practically 100 per cent were lymphocytes, a few "large mononuclears" being found. Bunting and Huston (1), in cannulating the thoracic duct of the rabbit, obtained a flow of lymph of 1 cc. in 10 minutes. They reported that they succeeded in ligating the lymphducts on both the right and left sides, after which they found, in one rabbit, the lymphocytes of the blood reduced to 310 per cubic millimeter. (The normal number in the blood-stream is around 3,000.) They pointed out the importance of the extensive lymphatic anastomoses about the entrance of the thoracic duct into the subclavian at the point of its junction with the jugular vein; and they mentioned also the anastomoses between the right and left sides, through the thymus, and the rôle of the right thoracic duct. They showed that the lymphocytes must leave the blood-stream very rapidly, to com-



pensate for the tremendous in-flow of lymphocytes through the lymph-channels, and they adduced evidence from which they concluded that these cells, without undergoing disintegration, leave the blood through the mucosa of the gastro-intestinal tract. Lee (4), ligating the thoracic duct in the cat, found the lymphocytes in the blood-stream reduced by 56 per cent with a return to the normal level in about 3 weeks. Minot and Isaacs (6) transfused a patient who had lymphosarcoma, and whose blood was very low in lymphocytes, with blood from another patient who had lymphoid leukemia; the immediate effect was a fourfold increase in the lymphocytes of the recipient's blood, but in 35 minutes they had almost, and in 2½ hours quite, fallen to their previous level.

Thorne and Evans (10) used the supra-vital method for studying the lymph of the thoracic duct of rabbits and report that they induced a monocytosis of 20 per cent by the intravenous injection of Niagara Blue. They conclude that, even if it is true that monocytes are produced in the lymph-glands, along with lymphocytes, their rate of production is insignificant; and that it is more probable that the few monocytes found in the lymph are, like the erythrocytes there, due to "the invasion of the lymph-current by some of the blood-elements."

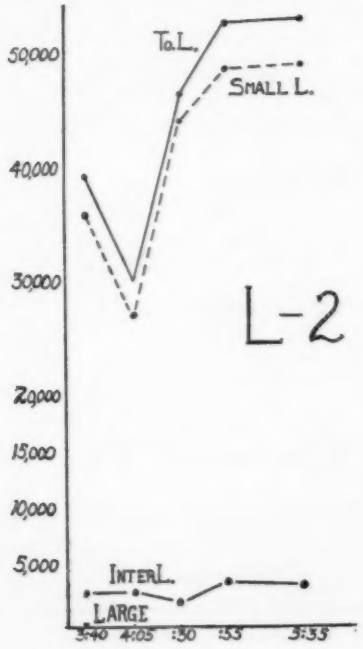


McJunkin (5) distinguished two types of cells of endothelial origin: The "non-rosette" cell, which lacks the peculiar rosette-like pattern of neutral-red granules when subjected to that stain, and the "rosette" cell, which possesses it. The rosette-cell he identified with Sabin's monocyte. Using both fresh tissues and paraffine-sections of supra-vitally stained tissue from normal and splenectomized rabbits, he

concluded that the rosette-cell arises in the lymph-nodes. He concluded further that the rosette-cell comes from the lymph-endothelium and that the non-rosette-cell comes from the blood-vascular endothelium.

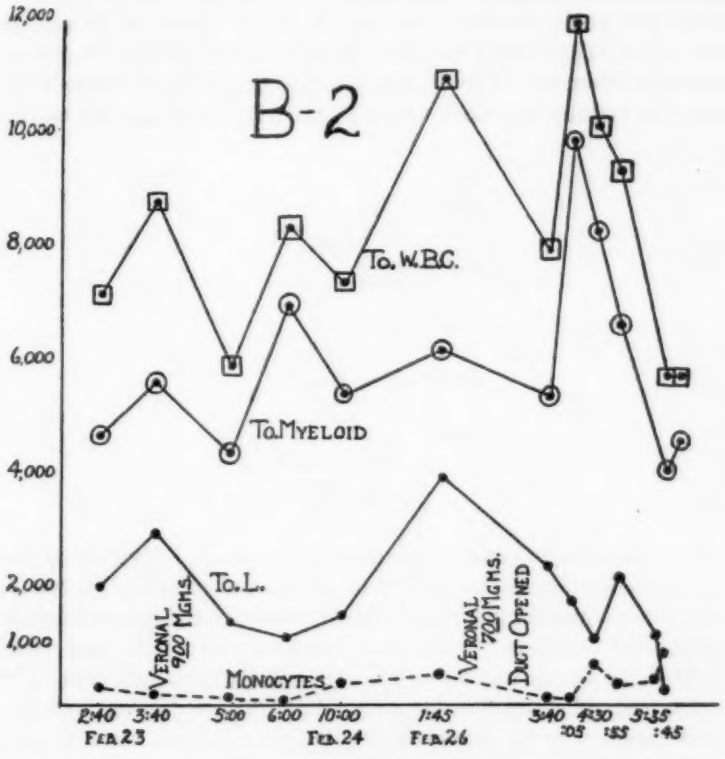
METHOD

The object of the work here reported was to get as representative a sample as possible of the rabbit's lymph just before it was discharged



into the blood-stream, and to subject it to a supra-vital study. As it was desired to have the results express as nearly as possible normal conditions, the choice of an anesthetic was carefully considered. Sodium-barbital (or "Veronal-sodium") was selected, and gave excellent results. It was given intravenously, 1 cc. of distilled water being used for each 100 mgm. of the drug. The optimal dosage was found

to be 300 mgm. per kilo of body-weight With this dosage, it took about 30 minutes for adequate narcosis to supervene. The drug as used did not cause complete unconsciousness, and hence a few whiffs of ether were given just before making the incision through the skin;

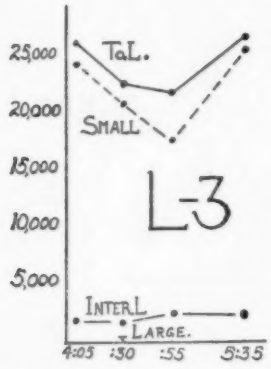


but for the rest of the operation, and through the observations, the sodium-barbital alone was sufficient, the animal remaining entirely quiet and placid.

OPERATION

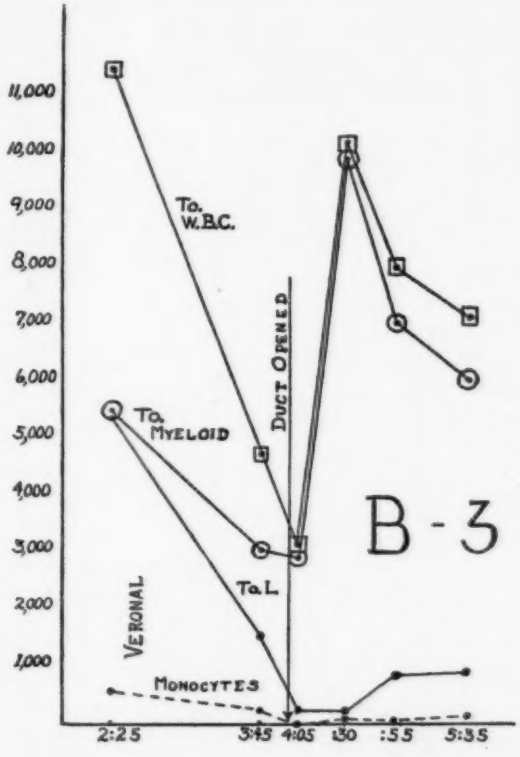
The left side of the chest, neck, and the left shoulder were clipped and shaved, and the rabbit was tied on the board, left side up, the

anesthetic having already been given. A pad, properly adjusted under the right shoulder of the animal, greatly improved the exposure of the operative field. The left fore-leg was drawn somewhat dorsally. No aseptic precautions were taken, the animal being sacrificed at the end of each experiment. The clavicle in the rabbit is imperfectly developed, being merely a very slender bone, tipped at its medial end with cartilage, which is connected with the sternum by the sterno-clavicular ligament. The clavicle can easily be palpated through the skin; the incision was made across it, extending from a point to the



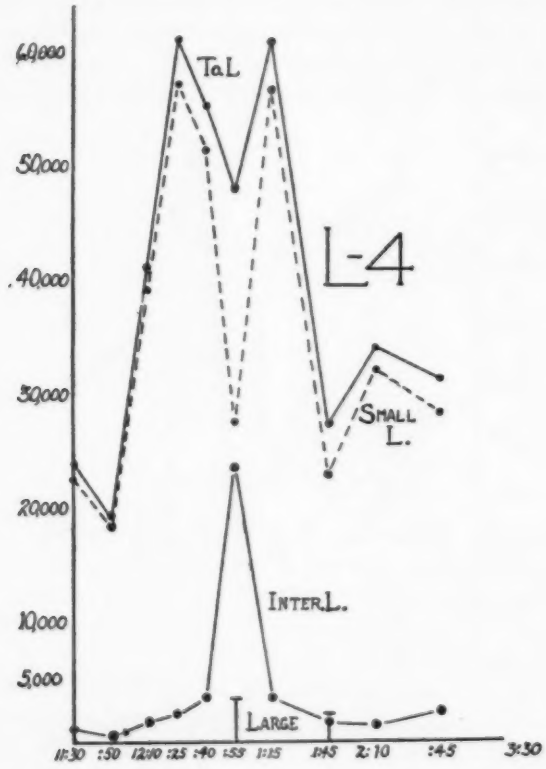
left of the sternum to another well up in the neck. Throughout the operation, hemostasis is of the first importance, in order that the lymph be not contaminated with blood; the operation can and should be made practically bloodless. After the platysma and fascia had been divided, the deeper muscles of the neck and shoulder were seen: The cleido-mastoid inserted on the middle of the clavicle; the basio-clavicularis inserted on the lateral third of the clavicle; and the four pectorals connecting the sternum with various parts of the clavicle or humerus. The muscles lying in the immediate field of operation were separated with blunt dissection, and one or two of them divided, so as to reveal the course of the external jugular vein. This vein was seen to dip beneath the clavicle. Associated with the clavicle was a large vein, which was tied and divided. The clavicle was then dissected free, and either drawn to one side or cut off. This revealed the

junction of the external jugular and subclavian veins, which is also the point of confluence of the thoracic duct and the lymph-vessels of the neck, just before they empty into the vein. This point of junction, as it lay in the bottom of the operative field, was easily recognized, for it is covered and bound down by a dense, white fascia, the apical-



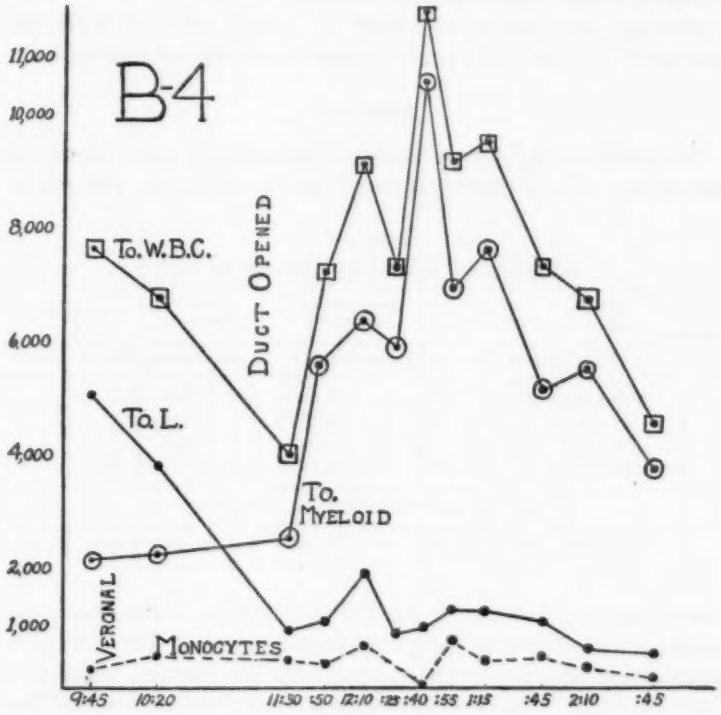
extension of the endothoracic fascia. In this region, extreme care was necessary, for in the rabbit the mediastinum is a very delicate and weak partition, and a unilateral pneumothorax is quickly fatal. It was by very careful dissection of this fascia that the lymph was reached. It was found best to use a very fine-bladed knife, going slowly, with good

illumination, and avoiding an occasional minute artery, the rupture of which would fill the lymph with blood. When the final wall was at last pierced, the lymph welled up, perfectly clear. Samples of it were then taken at will into a fine glass pipette, the mouth of which was placed at the point from which the lymph was escaping. A drop



of the lymph was forced from the pipette onto a cover-glass, which was then inverted on a slide having a film of neutral red, and rimmed with Salvoline in the usual way. Hemacytometer pipettes, for the total-cell-counts, were filled in the same manner. As the lymph continued to escape, it gradually accumulated in the operative cavity,

often clotting on the surface to form a thin, delicate, transparent pellicle. Occasionally, the lymph would clot at the point of exit, and the opening have to be freshened before the next sample was taken. The longest continuous period during which the flow of lymph was observed was a little over three hours; at the end of that time the



animal was still in excellent condition and there was no observable change in the rate of flow of the lymph.

Each time a sample of lymph was taken and a supra-vital preparation made of it, a count of its total cells was also made; and at the same time, a total cell-count was made of the blood (except in the case of Rabbit 1), and cover-slip preparations made for staining with Wright's stain, the blood being taken from an ear-vein. It would have

been preferable to have had the blood-differential made also from supra-vital films, but this would have demanded more assistance than was available. In the case of Rabbit 4, however, such assistance was available, and both the blood and the lymph were counted by the supra-vital method; and in addition, a total count of the red blood-cells was made at each sampling in this animal.

Although eight rabbits were used, the results were very much the same in all, and the data from four are here reported as being typical.

RESULTS

The results of the present study are presented in eight charts and two tables. The B-charts represent the blood-picture, and the L-charts the lymph-picture in each of four rabbits. The charts and tables, being self-explanatory, need little comment. The basophiles, eosinophiles, and polymorphonuclear cells are grouped together as "total myeloid." In chart B-1, the monocytes were so few that they could not conveniently be shown. The large lymphocytes, occurring at irregular intervals, are indicated by isolated vertical lines rising from the base-line. In Chart B-1 the blood-picture is shown in percentages, while in all the other charts the absolute numbers are given.

TABLE I

Average numbers of the various cell-types in the lymph

ANIMAL	TOTAL WHITE BLOOD CELLS	SMALL LYMPHOCYTES		INTERMEDIATE LYMPHOCYTES		LARGE LYMPHOCYTES		MONOCYTES	
		Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number
1	20,466	84	17,291	14	2,865	1	204		
2	44,960	92	41,363	7.3	3,282	0.1	44		
3	24,270	90	21,843	9.2	2,233	0.5	121		
4	40,728	89	36,247	7.8	3,176	1.9	773	0.15	61
Average of all the animals taken together									
	32,606	*88*	*28,693*	*10*	*3,260*	*0.8*	*260*	*0.03*	*15*

It will be seen in all the charts that, immediately following the injection of the anesthetic, there is a temporary fall in the leucocytes,

TABLE II

Average numbers of the various cell-types in the blood stream
Before and *after* opening of the lymph-duct

ANIMAL	TIME	TOTAL WHITE BLOOD CELLS	MYELOID		TOTAL LYMPHOCYTES		SMALL LYMPHOCYTES		INTERMEDIATE LYMPHOCYTES		LARGE LYMPHOCYTES		MONOCYTES		RED BLOOD CELLS
			Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	
1	Before		62		37		26		11		0		1		
	After		*89*		*9.2*		*6.7*		*2.2*		*0.25*		*1*		
2	Before	8,128	69	5,588	28	2,265	18	1,521	8.2	716	0.3	24	3.1	260	
	After	*8,640*	*77*	*6,770*	*15*	*1,329*	*9.6*	*882*	*5.6*	*483*	*0*	*0*	*7.2*	*622*	
3	Before	6,466	68	4,396	29	1,875	19	1,228	8.3	596	0.88	56	3.3	213	
	After	*8,440*	*90*	*7,596*	*8.8*	*742*	*7.3*	*616*	*1.3*	*103*	*0*	*0*	*1.3*	*103*	
4	Before	9,586	42	4,026	49	4,697	43	4,121	4.3	412	1.6	153	8	766	7,043,000
	After	*8,197*	*81*	*6,639*	*14*	*1,147*	*11.2*	*918*	*2.4*	*196*	*0.5*	*41*	*5.5*	*450*	*7,280,000*
Average of all the animals taken together															
	Before	8,060	60	4,670	33	2,945	26	2,290	5.2	574	0.8	77	4	379	
	After	*8,428*	*84*	*7,001*	*11.7*	*1,072*	*8.7*	*805*	*2.8*	*290*	*0.18*	*41*	*3.7*	*391*	

followed by a recovery. Whether this fall is a direct effect of the drug used cannot be determined from the data now at hand. In this connection, the case of Rabbit 2 is interesting. This animal was given an over-dose of the anesthetic, but not then operated upon; two days later it was given a smaller dose, followed by operation.

The tables give the average of all the counts in each animal, and the final averages of all the animals taken together. The decimal places, except in the smaller numbers, have been disregarded, as not being significant.

At the end of the experiment, Rabbit 4, a mesenteric lymph-gland was punctured and aspirated with a capillary pipette. The fluid thus obtained was studied supravitally with the following results:

	per cent
Small Lymphocytes	88
Intermediate Lymphocytes	10
Large Lymphocytes	1
Clasmatocytes	1

The striking agreement with the average of the counts of the cells in the lymph-stream is at once apparent (See Lymph Table).

That the lymph in those observations was uncontaminated by blood is indicated by the fact that in only 4 preparations were any red blood-cells to be seen, and in those four, only a very few were noted. In one preparation, a polymorphonuclear leucocyte was seen. None of the other preparations showed any cell-element peculiar to the blood-stream.

SUMMARY

Normal rabbits were anesthetized by the intravenous injection of sodium-barbital in distilled water, and operated upon so as to expose the lymph-vessels of the neck where they empty into the venous system. The point of confluence of these lymphatics with the thoracic duct was opened, and the lymph subjected to a supra-vital study of its cells. These cells were found to average about 32,000 per cubic millimeter, and to be practically all lymphocytes. No evidence was found to support the idea that monocytes are produced anywhere in the lymphatic apparatus.

CONCLUSIONS

1. The cells of the lymph of the rabbit, under the conditions of the experiment, are practically all lymphocytes, the average total number being 32,606 per cubic millimeter, of which Small Lymphocytes constitute about 88 per cent, Intermediate Lymphocytes about 10 per cent and Large Lymphocytes, at irregular intervals, less than 1 per cent.

2. Occasionally a monocyte can be found, but so rarely as to indicate that this cell-type is not produced by the lymphopoietic tissues, but is rather a chance and infrequent invader of the lymph-stream.

3. Opening the lymph-channels on the left side alone, just at the point where they discharge into the neck-veins, markedly reduces the number of lymphocytes in the blood, the average being a reduction of about 66 per cent. The lowest observed lymphocyte-count was 204, or 2 per cent (Rabbit 3).

4. The relative numbers of the cell-types obtained by puncture of a lymph-gland show a striking agreement with those in the lymph-stream.

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INFANTILE RICKETS

Treatment by Intramuscular Injection of a Cod-Liver Oil Concentrate[1]

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AND

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Adequately controlled clinical observations and numerous animal experiments testify to the efficacy of cod-liver oil in the prevention and cure of rickets. The manner in which this agent acts, however, is not at all clear. Metabolism experiments show that after the prolonged administration of the oil, a negative calcium and phosphorus balance may be converted into a positive one. The improvement in the retention of calcium and phosphorus is associated with a diminished excretion of these elements in the feces (1). This has been interpreted as indicating that the beneficial effect of cod-liver oil is due to its action upon the gastro-intestinal tract.

Findlay (2) agrees with Lesne and Vagliano (3) that cod-liver oil is without effect on rickets when injected subcutaneously; and Zucker and Matzner (4) failed to produce healing in rachitic animals with subcutaneous injections of an active extract of cod-liver oil. On the other hand, Soames (5) protected rats against rickets by the parenteral administration of cod-liver oil, and Stepp (6) has reported the cure of experimental rickets following the intraperitoneal administration of this remedy. Hess and his associates (7) showed that irradiated cholesterol protects rats against rickets when injected subcutaneously.

Shortly after Zucker's preliminary report (8) on the preparation of an anti-rachitic extract containing the unsaponifiable material of cod-liver oil, we began a series of experiments, using a concentrate of the

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active fraction of the oil. We administered this fraction intramuscularly in two cases of active rickets.

Cod-liver oil was saponified by means of alcoholic potash and extracted with petroleum ether. The combined petroleum ether extracts, which consisted largely of cholesterol, were shown by feeding experiments on rats and infants to possess practically the full therapeutic value of the original oil. No attempt was made to separate the inert cholesterol. The combined petroleum ether extracts, made up to the volume of the original oil, were equivalent volume for volume to the original oil. One cubic centimeter of this petroleum ether solution is termed in this report a therapeutic "unit" and is equivalent to 1 cc. of cod-liver oil. The dose to be administered was measured into a dish and the petroleum ether allowed to evaporate in the air. The solid residue, redissolved in ethyl ether, was injected into the gluteal muscles of the patient.

Case 1. S. C. a colored female, age 9 months, was admitted to The Harriet Lane Home on June 23, 1924, for acute secondary pneumonia (following measles) and otitis media. She was poorly developed and poorly nourished. In addition to the respiratory infection, there was marked evidence of rickets. The head was large, the parietal and frontal bosses prominent, and craniotabes was present. There was a marked rachitic rosary and a prominent Harrison's groove. The ribs were flaring and the chest sunken laterally. The epiphyses at the elbows, wrists, knees and ankles were enlarged. The tibiae were bowed. The *radiogram* showed marked active rickets. The *blood serum* on June 30th contained 8.3 mg. of calcium and 2.9 mg. of phosphorus per 100 cc., giving a Ca × P product of 24.[2]

Treatment. The first dose of 25 units of cod-liver oil concentrate was given July 1. Four days later there was no change in the concentration of the serum phosphorus. The second dose was administered July 8th. On July 12th the serum calcium had risen to 10.6 mg. and the inorganic phosphorus to 4.2 mg. per 100 cc. (a product of 44). The third dose, given July 15th, was followed by a still greater increase in the phosphorus concentration, which, on July 22nd, was 6.2 mg. per 100 cc. of serum, thus raising the Ca × P product to 64. Coincidently with this change in the concentration of the calcium and inorganic phosphorus of

[2] This product is obtained by multiplying the concentration of the calcium by the inorganic phosphorus concentration, each expressed in milligrams per 100 cc. of serum. When the product is below 30 to 40, active rickets is invariably present. In healing, when the product exceeds these figures, calcium salts begin to be deposited in the bones (Howland, J., and Kramer, B.: Factors Concerned in the Calcification of Bone. *Proc. Am. Pediat. Soc.*, 1922, xxxiv, 204.)

the serum the radiograms of the bones showed marked progressive deposition of calcium salts along the epiphyseal lines. Throughout the course of treatment the child received a diet of buttermilk and farina, which was well taken. The progress of healing is summarized in the following table:

DATE	TREATMENT (C.L.O. CONCENTRATE)	SERUM			RADIOGRAM
		Ca	P	Ca × P	
		mg. per 100 cc.	*mg. per 100 cc.*	*mg. per 100 cc.*	
June 24					Rickets
June 30		8.3	3.0	25	
July 1	25 units				
July 5			2.9		
July 8	25 units				Rickets
July 12		10.6	4.2	45	
July 15	25 units				
July 22		10.4	6.2	64	Healing rickets

Case 2. R. G., colored female, age 9 months, was admitted to The Harriet Lane Home on June 10, 1924, for pertussis, secondary pneumonia and otitis media. For three days prior to admission she had had convulsions. In addition to the evidences of broncho-pneumonia, the child showed marked signs of rickets. The head was large, with prominent frontal bosses and slight craniotabes. Beading of the costo-chondral junctions and a Harrison's groove were noted. The epiphyses at the wrists were enlarged. The Chvostek and Trousseau signs were absent.

Electrical reactions were C.C.C., 1.4 ma.;[3] A.O.C., 3.2 ma.; A.C.C., 2.2 ma.; C.O.C., 5 ma.

Blood serum. Calcium 6.8 mg. per 100 cc. of serum. Phosphorus 4.2 mg. per 100 cc. of serum. Ca × P product 28.

The *radiogram* showed marked active rickets.

These findings, together with the history of convulsions, indicate that this was a case of rickets complicated by tetany.

Treatment Sodium luminal was given as a sedative and the administration of calcium chloride was begun on June 25th and continued for one month. On June 24th, 20 units of cod-liver oil extract were given intramuscularly. On June 30th the serum calcium had risen from 6.8 to 10.2 mg. and the inorganic phosphorus had decreased from 4.2 to 2.7 mg. per 100 cc. but the Ca × P product remained the same (28). During this period the child had been receiving calcium chloride by mouth and the increase in the serum calcium was due to this drug. There was as yet no curative effect of the cod-liver oil demonstrable. The second dose of 25

[3] ma. = milliamperes.

units was given on July 1st, but on July 5th the serum phosphorus was still 2.9. The third dose of 25 units was given July 8th. Radiograms of the bones on that day, showed beginning deposition of calcium salts. On July 12th the inorganic phosphorus of the serum had risen to 3.1 mg. per 100 cc. making a Ca × P product of 33. The fourth dose of 25 units was given July 15th. On July 21st the calcium was 10.2 and the phosphorus 5.5 mg. per 100 cc., or a product of 57. The radiogram revealed that complete healing had taken place in the bones.

During the entire period of treatment the child received a diet consisting only of a milk mixture and farina, a diet which of itself has no antirachitic effect. The appetite was fair and there were no periods of starvation.

DATE	TREATMENT (C.L.O. CONCENTRATE)	SERUM			RADIOGRAM
		Ca	P	Ca × P	
		mg. per 100 cc.	*mg. per 100 cc.*	*mg. per 100 cc.*	
June 22	$CaCl_2$	6.8	4.2	29	
June 24	20 units				
June 26					Marked rickets
June 30		10.2	2.7	28	
July 1	25 units				
July 5			2.9		
July 8	25 units				Healing rickets
July 10					Healing rickets
July 12		10.8	3.1	33	
July 15	25 units				Healing rickets
July 21		10.2	5.5	56	Healed rickets

COMMENT

The usual treatment of rickets consists in the administration of 5 cc. of cod-liver oil by mouth three times a day. Under these conditions, the phosphorus and the calcium of the blood serum rise to normal levels (Ca × P product = 40 to 60) in 2 to 3 weeks, and in about 3 weeks the first signs of deposition of lime salts appear in the radiograms of the bones. The two cases reported received a concentrate of cod-liver oil by the intramuscular route at weekly intervals, in doses equivalent to 25 cc. of the oil. In the first patient, with the usual type of low-phosphorus rickets, the serum phosphorus rose to a normal level shortly after the second intramuscular injection, twelve days from the beginning of treatment. A third dose was given on the 15th day. By the 22nd day the phosphorus had risen to the high value of 6.4 mgm. per 100 cc. and the radiograms showed advanced

healing. The second patient had a low calcium as well as a low phosphorus concentration in the blood serum and was therefore a case of "border line tetany." For this reason, calcium chloride was administered by mouth. The cure of the rickets in this patient cannot be attributed to the calcium chloride. This salt, if administered in large amounts, will maintain an approximately normal concentration of serum calcium but unless supplemented by an anti-rachitic agent, such as cod-liver oil, produces an actual decrease of the inorganic phosphorus of the serum and thereby prevents healing of the rickets. The delay in the rise of the inorganic phosphorus concentration in the serum in this case must probably be attributed to the calcium chloride. The subsequent increase in the concentration of phosphorus in the serum speaks for the potency of the concentrate.

CONCLUSION

Two children with active rickets were treated with an ether solution of a concentrate of cod-liver oil, injected intramuscularly. Fresh calcification of the rachitic cartilage appeared within three weeks. The changes in the concentration of calcium and inorganic phosphorus of the serum were identical with those previously observed following the oral administration of cod-liver oil.

Whereas cod-liver oil increases the absorption of phosphorus and calcium from the intestine (1), the fact that it is effective when administered intramuscularly indicates that this is not purely a local action upon the intestinal mucosa but is a general one and exerted through the circulation.

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BOOK REVIEWS

Diseases of the Nose and Throat. By Sir St. Clair Thomson. 3rd Edition. 943 pp. $14.00. (*D. Appleton & Co., New York, 1926.*)

The author, in a very pleasant manner, has incorporated his mature scientific and clinical experience into the present volume.

As stated in the preface, much obsolete material has been deleted and the space thus gained has been utilized to discuss newer ideas and methods: for example, Malignant Disease of the Larynx and the masterly one-stage operation developed for its cure. It would be difficult to get a more comprehensive broad-minded discussion of Nasal Polypus in so short a space.

The Table of Contents gives a very clear idea of the general arrangement and makes it easy to locate any particular subject. There is an admirable index which facilitates looking up detailed information. The references to Original Sources are well chosen and from a wide range.

Numerous carefully selected illustrations with good legends facilitate a proper visualization of the text. The attention given to Bacteriology and Pathology as well as to Anatomy leads to a sounder conception of the different subjects and makes for a broader understanding of diseased conditions.

The medical profession, laryngologists and medical students will surely accord a hearty welcome to this long anticipated edition of a most useful book.

H. R. S., Jr.

The Duodenal Tube. By Max Einhorn. Second edition, revised and enlarged. 206 pp. $3.00. (*F. A. Davis & Company, Phila., 1926.*)

In his inimitable style Dr. Max Einhorn has written a book entitled "The Duodenal Tube." Furthermore, he has just brought out a second edition. In two hundred pages, many of them covered with illustrations, he tells of its history, development, and usages.

He lays considerable stress on its value as a diagnostic medium—*viz.*, in recovery of duodenal contents, their analysis and interpretation. The methods he advises for the estimation of pancreatic enzymes have the advantage of age. He evidently does not believe the newer methods worthy of note.

Considerable attention is paid to the analysis of the bile and the diagnostic significance of biliary sediment. He reproduces a remarkable table showing color reactions in relation to types of gall-bladder disease. No attention is paid to the effect of hydrochloric acid and other gastric contents upon these changes.

As an aid in treatment the tube doubtless has its place, especially in the post-operative administration of fluids and nourishment.

Even the most ardent admirer must admit that the last third of the book delves

a bit into the realms of imagination and serves to make diagnoses difficult rather than easy. When there is a direct method either in history, x-ray, or physical examination, it would seem superfluous to let a tube lie for 48 hours in the stomach to demonstrate a pyloric obstruction.

Among some of the instruments described are the pyloric dilator, the double balloon pyloric dilator, the duodenal obturator, the intestinal tube used for upper colonic irrigations after the tip has reached the caecum, and the intestinal delineator, made of braided silk and annealed copper, to diagnose cardiospasm, pylorospasm and to be x-rayed in its passage through the intestine. That he can induce patients to submit to its recovery *per rectum* is a tribute to his control.

L. M.

Biological Relations of Optically Isomeric Substances. By Arthur R. Cushny. 80 pp. $2.00. (*The Williams & Wilkins Co., Baltimore, 1926.*)

This is the third series of lectures given at the Johns Hopkins Medical School under the Charles E. Dohme Foundation and comprises some eighty pages of very readable matter. Optical Isomers or Stereoisomers have hitherto been of interest chiefly to the chemist and more particularly to the specialist in organic chemistry. In the present treatise the importance of the subject is brought home to the physiologist, pharmacologist and general biologist. Even from a chemical point of view the existence of two or three compounds identical in composition and differing only in the spatial relationship of their constituent atoms is a most remarkable phenomenon. It is little short of amazing to learn that such very closely related compounds may actually exhibit definite and decided differences in physiological and pharmacological action.

The series begins with a brief historical sketch reviewing the early and fundamental work by Biot, Pasteur and others. The next chapter takes up the relation of Enzymes to optically active bodies. This is followed by two important chapters dealing with the decomposition of Isomers in living tissues and the pharmacological action of Optical Isomers and related phenomena to which Cushny has devoted his later years.

As is true of all collected publications, the work, while reasonably up to date, does not include some of the latest developments in this field. Thus, for instance, no mention is made of the interesting findings in regard to the difference of physiological action of Isomers in the field of Phyto-pharmacology, where it has been shown that a similar difference in response is exhibited by plants as well as by animals. Again, the present lecture being a somewhat dogmatic review of work along lines in which Cushny was specializing, it is not surprising that he is found to disagree with some of the experiences of other investigators, as for instance is exemplified in the study of the pharmacological effects of different camphors. The author's subject is a very complicated one and in the reviewer's opinion one which undoubtedly will play a very important rôle in the development of the pharmacology and chemistry of the future. It may be well to conclude with a

quotation from an address by Cushny published elsewhere, to emphasize the importance of this thought.

"This optical activity is, in fact, the most persistent evidence of life which we possess. An optically active alkaloid or acid may be kept for centuries after the plant which formed it and the chemist who isolated it are dead, but it will still possess its optical activity, testifying that it was formed by some living thing either directly or indirectly. When we find an optically active substance in the earth, we may know at once that it arose through the agency of life. The petroleum we burn, for example, must have arisen from living tisues, for it is optically active. Not only is it the most persistent sign of life, but it is the most definite physical characteristic of life. No other can be measured in actual numbers in the same way."

D. I. M.

Lead Poisoning. By Joseph C. Aub, Lawrence T. Fairhall, A. S. Minot and Paul Reznikoff. Medicine Monographs. Vol. VII. 265 pp. $4.00. (*The Williams & Wilkins Co., Baltimore, 1926.*)

This is the seventh in the series of Medical Monographs and is in reality a bound reprint of a paper published by the authors in the quarterly periodical *Medicine.* It constitutes an important and timely contribution to the study of what is perhaps the most important and most common form of industrial poisoning in this country, and deals with every aspect of the subject. Beginning with an historical résumé the authors discuss thoroughly the detection of lead in organic material and the estimation of its amount. The physico-chemical behavior of lead compounds and their transportation and deposition in the body are then dealt with. The very important subject of distribution and storage of the metal in the organism and its absorption and excretion deservedly receive the consideration of six chapters in the book. The pathological changes that result from lead poisoning are described adequately in chapter X, in which the pathological anatomy and histology are given. The effects of lead on the blood cells which might properly come under the heading of pathology are included in Part III, which deals with the physiology of lead poisoning, and the blood changes are discussed at great length, as might be expected from the extensive experience of the authors in this particular field. Injuries to the germ cells are also touched upon. Interesting information on the pathology and pathological physiology of the lead line and lead colic as well as on changes in the nervous system are described. Part IV deals with the clinical aspects of the subject and discusses diagnosis and treatment. Last but not least, the book includes a bibliography of some five hundred references which completes perhaps the most valuable monograph on lead poisoning which has as yet been published.

D. I. M.

An Atlas of Human Anatomy. By Carl Toldt. Translated by M. Eden Paul. Revised ed. 2 vols. 985 pp. 1506 ills. $10.00. (*The Macmillan Co., New York, 1926.*)

The translator's prefatory remarks are strangely out of keeping with the contents of this remarkable atlas. At once we are told that the science of human anatomy is closely verging on finality. And yet we have only to turn the pages of the atlas to find a rich provision of figures from foetal and infantile life inviting us to further question. Also, the field of anatomical variation, usually neglected in the textbooks, is here treated with proper dignity; for instance, we see in pictures, what we must have seen occasionally in the laboratory, the sacrum partially fused to the fifth lumbar vertebra or to the coccyx; or again, we see the sternalis muscle, the axillary arch and the epitrochleo-anconeus muscle; elsewhere the common mesentery of the ascending and transverse colons; even that rather rare condition, the persistent left superior vena cava. Among other commendable features of the work are an illustrated classification of joints and an exhaustive illumination of the inguinal and femoral regions.

Defects, amounting to awkwardness, however, are not lacking. The meticulous effort to adapt English, American, and International terminology, all in one, to the labelling may well serve the advanced student, but is certain to annoy the beginner, whose first difficulty with anatomy is usually one of vocabulary. Also, in many figures the reference pointers are hard to follow. As for the figures themselves, the only ones that might be called actually bad are those of the petrous portion of the temporal bone. There the technical difficulties have not been met. It may be questioned, too, whether the illustrations showing gelatine injections of the subarachnoid spaces are entirely adequate.

On the whole, it should be said that the new Toldt atlas combines very unusual dissection with excellent illustration, these transmitted by a translator and a publisher who have spared themselves nothing toward the work's success. With such a book as guide, and after overcoming the necessary trials of vocabulary, the student should follow the discipline of anatomy in a high spirit of inquiry and exploration.

C. F. D.

Enzymes. Properties, distribution, methods and applications. By Selman A. Waksman and Wilburt C. Davison. 364 pp. $5.50. (*The Williams & Wilkins Company, Baltimore, 1926.*)

It is a matter of growing importance that our somewhat static conception of biochemistry should be enlivened by a fuller knowledge of enzymes. It is a fact not sufficiently familiar to the profession that the activity of these mysterious agents underlies and actually constitutes the total of bodily change. Our microcosm is not only an example of biochemical reaction in the commonly conceived sense, but it also represents a vast series of equations in which an unknown must be symbolized on both sides of the arrows. This symbol represents the biological

catalyst, itself a product of living cells and a *sine qua non* of organic being. These enzymes—the true anabolists, the true catabolists, throttled or released by bombardment of hydrogen atoms—how little indeed have we reflected upon them! Zymology, from Van Helmont to the present day, has maintained a technical aloofness, has run an uneven course and left a scattered literature. The double virtue of the present book lies in the fact that it integrates, and, by so doing, familiarizes. It is an admirable, critical review of our knowledge of enzymes. The confusing array of data, gathered from all branches of the subject, are here arranged intelligibly in four major sections,—the properties of enzymes, their distribution, their preparation and study, and finally, their practical applications in the arts and sciences. Methods have been described, usually with the authors' preferences. Very little space is devoted to theory. The reader may at times wish that a more expanded description had been made of certain procedures, but this would have rendered the book unwieldy. Instead, literature references—of which 2,000 are to be found—provide for more intensive reading. Waksman and Davison have produced the most exhaustive monograph that exists on the subject in the form of a concise, practical and critical résumé. It will prove enlightening to any reader and, we should judge, invaluable to the research worker in its own, as well as in other fields.

B. S. C.

2

SKIN REACTIONS TO HUMAN SERA[1]

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The earliest record of the toxicity of normal blood is to be found in the report of the Royal Philosophical Society in 1666. From that date to the present time investigations have been carried on relative to this problem; and de Kruif (1), in his publication in 1917, has summarized the literature dealing with the toxicity of normal serum. His investigations with Novy (2) were carried on by animal experimentation with reference to anaphylaxis. They concluded that before and during the coagulation of normal blood a substance, anaphylatoxin, is found, which is responsible for the toxicity of sera at room temperature, and that, especially at 55° to 60°C., the specific shock in anaphylaxis is the result of anaphylatoxin production, the indirect result of the union of antigen and specific antibody. They did not study the local reactions produced by the intracutaneous injections of human toxic sera into human skin.

The object of the experiments to be described was to acquire some knowledge of the reactions which might occur in the human skin after an injection of unheated homologous and of heterologous sera; to learn something of the intradermal reaction to heated homologous and heterologous sera; and also to study skin reactions from sera aged in the ice-box.

The blood was taken from the vein under sterile precautions and was put into sterile centrifuge tubes. It was allowed to stand at room temperature for from one-half to one hour, so that a firm clot was formed. This was loosened with a sterile pipette. The tube was covered with a piece of sterile gauze and centrifuged for about three quarters of an hour at good speed. The serum was immediately pipetted off into sterile tubes, which were always kept in the ice-chest at a temperature of about 4°C. The heating was done in a constant water-bath—usually standing at 56°C. but occasionally at 54° or 55°C. The interval between the

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bleeding of the patient and the re-injection of the serum was usually one or two days; in a few cases six to nine days elapsed between the two procedures.

Unheated homologous serum. The first series of experiments was carried out on twenty individuals. Five of these were sensitive to pollens. In the great majority of these subjects there was practically

TABLE I

CASE NUMBER	UNHEATED SERA		HOMOLOGOUS SERA HEATED AT 56°C.												INTERVAL BETWEEN BLEEDING AND TESTS
			10 minutes		20 minutes		30 minutes		40 minutes		60 minutes		120 minutes		
	W	R	W	R	W	R	W	R	W	R	W	R	W	R	
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	days
1	0.8	1.4			1.1	3.2	1.1	3.3	1.3	3.6	1.4	4.4	1.7	7.0	0
2	0.5	0.5	0.5	0.5	0.5	0.5	1.0	4.0	1.1	4.8	1.6	5.7	1.8	6.5	1
3	0.6	1.9							1.4	5.0			1.4	4.75	9
4	0.5	0.5							1.3	3.5			2.2	4.5	3
5	0.8	1.5							1.0	3.25			1.0	4.0	3
6	0.9	1.7			1.0	1.6	1.2	2.4			1.25	3.1	1.4	3.6	1
7†	1.2	1.8			1.2	1.5	1.4	2.2	1.3	2.0	1.6	3.0	1.6	3.4	5
8	0.8	1.1			0.8	1.0			1.0	1.2	1.1	1.4	1.3	3.1	2
9	0.95	1.2							1.2	2.5	1.25	2.6			1
10	0.8	1.7	0.5	0.5	0.5	0.5	0.8	1.6	1.4	4.0	1.0	2.5	1.0	3.0	3
11	0.9	2.1					1.2	4.2	1.15	4.2	1.2	2.9	1.25	3.2	2
12†	1.0	2.6							1.2	3.8			1.45	4.5	7
13†	1.1	2.0					1.4	2.5	1.7	2.5	1.5	2.2	1.5	2.0	2
14	0.85	3.3			1.15	4.2	1.05	5.3	1.15	5.0	1.25	7.0	1.2	5.5	1
15	0.9	1.8			1.4	4.0	1.5	4.5	1.5	4.5	1.3	4.0	1.4	5.0	7
16	0.6	2.7			0.6	2.5	0.9	3.5			1.0	4.0	1.4	4.0	2
17	0.9	0			1.1	1.3	1.4	1.5	0.8	1.0			0.8	0.8	4
18†	1.2	2.4			1.4	2.0	1.3	2.8			1.5	2.8	2.1	2.8	2
19	0.85	1.05			1.25	1.45	1.2	1.9			1.3	2.6	1.0	1.25	2
20	0.9	1.2					1.1	3.5			1.6	4.5	1.7	4.3	2

W = wheal, R = redness.
† Reaction to own serum (see text).

no reaction to the intracutaneous injection of 0.1 cc. of their own untreated serum. The wheal at the end of the injection varied from 0.7 to 0.9 cm. in diameter and in most cases it did not increase beyond these limits. Any slight redness that appeared was pale in color and covered only a small area. In four individuals there were slight reactions (these are marked with a dagger in Table I). This injec-

tion of the unheated serum was always made on the palmar surface of the forearm near the elbow, which is the most sensitive skin area of the arm and where the largest reactions are normally elicited. The other injections of the serum heated at 56°C. for increasing lengths of time were made at intervals on the palmar surface of the forearm. In a series of injections the longer the serum was heated, the further away was the site of injection from the elbow.

Homologous serum heated at 56°C. In every one of these 20 cases so treated, the patient's serum, after having been heated at 56°C. for thirty minutes or more, evoked a decided reaction on intracutaneous injection. This reaction included both a central wheal and a surrounding erythema. The wheal and erythema were at their maximal size 15 minutes after the injection and were fading within a period of 30 minutes. After an hour and a half all signs of the reaction were gone with the exception of a small area of redness around the site of the puncture. When the heated serum was injected, the subject often complained of a burning sensation in the area covered by the wheal and of an itching of the skin around it. These sensations came on during the injection and usually lasted less than five minutes. When the wheals were very large, pseudopodia were seen. There was considerably less redness in proportion to the size of the wheal than is shown in individuals giving positive reactions to the intracutaneous injection of pollen extracts, but in other respects the reactions were exactly similar.

In two-thirds of the patients who were tested with serum heated at 56°C. for only 20 minutes, a positive reaction was obtained. When the heating was continued for 30 minutes every individual tested, with the exception of one, gave a marked reaction. In this one exception the heating had to be of 40 minutes' duration before the serum provoked a reaction. In every case the heated serum gave rise to reactions that were much more marked than any reaction to untreated serum. In the majority of individuals the maximal reaction appeared after the serum had been heated for two hours, which, in most cases, was the maximal time of heating. In one instance the serum was heated for 3½ hours, but even after that length of time it produced about the same reaction as a specimen of the serum which had been heated for one hour. In general the longer the serum

was heated, the larger was the reaction—at least, up to two hours' heating. In one case the maximal reaction occurred after 30 minutes' heating. In seven the maximal reactions occurred after heating from 40 to 60 minutes. In these cases further heating resulted in a serum that caused less marked reactions.

If it had been possible to make all the injections in skin areas that were equally sensitive, the difference between the reactions to the unheated and heated serum would have been considerably more marked.

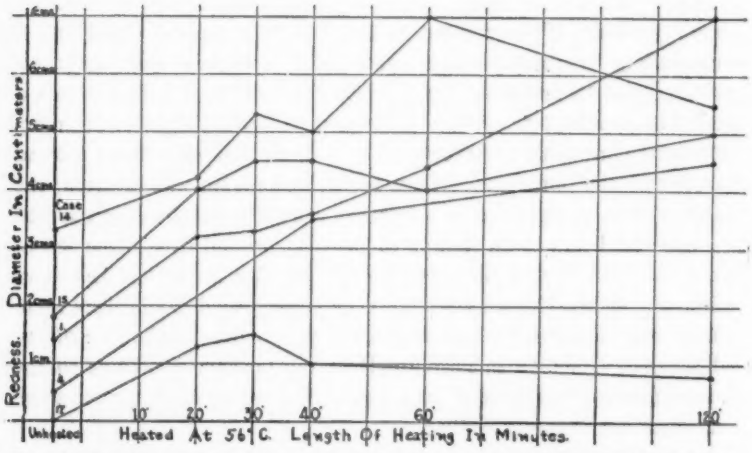

FIG. 1. CHARACTERISTIC CURVES SHOWING THE AREAS OF REDNESS OCCURRING IN THE SKIN OF INDIVIDUALS INJECTED WITH HEATED AND UNHEATED SERA

The effect of aging of the serum at ice-chest temperature. Samples of serum first tested on a number of individuals were kept in the ice-chest. After we had tried out their reactions to their own sera heated and unheated, we put the serum samples away in the ice chest, and after an interval of time the specimens were tested again on the same individual. The injections were spaced down the arm in the same order as before, but care was taken to choose fresh skin areas. After standing at ice-chest temperature for a month, the *unheated* serum caused a marked reaction, almost as great as that produced in the first tests by the serum heated to 56°C. for two hours. When

the *unheated* serum was allowed to stand in the ice-chest for only five days before the second injection was made, the second reaction was practically the same as the first. After an interval of twelve days the reaction to the second injection was somewhat larger than to the first. In one individual, when the second injections were made after a period of sixteen days, a marked reaction was given to the *unheated* serum. Apparently, after twelve days' standing in the ice-chest some change begins to take place in the serum. This change is more marked after intervals of sixteen and thirty days. We have not determined what interval would produce the maximal change.

As a general rule, when the *heated* serum was allowed to stand in the ice-chest for 12 days, it became considerably less irritating; in other words, the reactions elicited on second injection were less than when the heated serum was injected into the skin within that period.

The effect of heating homologous serum at different temperatures. We attempted to find out if the serum would cause similar reactions when heated at temperatures other than 56°C. When it was heated at 37°C. for one hour the reaction was practically the same as that given by unheated serum. In fact, the reactions tended to be a little less marked than those from unheated serum. When the sera were heated at this temperature for two or three hours, injections gave rise to small reactions, which were a little larger than the "unheated" reactions, but much less marked than the "56°C." reactions.

Heated at 48° and 50°C. the sera caused more marked reactions than when heated at 37°C., but they were still considerably less marked than those produced by serum heated at 56°C.

In one case where the undiluted serum was heated at 60°C. for an hour, the reaction was smaller than that produced when it was heated at 56°C. It was also smaller than the reaction from serum heated at 50°C. for one hour.

It appears from these results that after the serum is subjected to varying degrees of heat, from 37° to 56°C., for a sufficient length of time, it becomes irritating when injected into the layers of the skin. However, the temperature between 54° and 56°C. seem to make the serum much more irritating.

Heterologous human serum. These experiments were carried out on 17 individuals, but in the majority of cases only three tests were

made on each, namely: (1) Serum unheated; (2) serum heated at 56°C. for 30 minutes; (3) serum heated at 56°C. for two hours. In practically all cases the reaction to heterologous unheated serum was slightly greater than the reaction to homologous unheated serum. The reactions to their own heated serum and to similarly treated serum of another individual were nearly equal. In some cases the reactions to heterologous serum were somewhat more marked than those produced by the homologous; in others somewhat smaller. Here, also, the reaction to the heated serum was greater than that to the unheated, except in one case where there was no difference between the two.

Experiments with guinea-pigs. The serum obtained from guinea-pigs, heated for two hours at 56°C. and then injected intracutaneously into the same animal did not cause any reaction.

Guinea-pig serum both heated and unheated was injected intracutaneously into two human subjects. In these experiments the largest reaction was obtained with the unheated serum. With heated guinea-pig serum the longer heated specimen caused more response than one that had been heated for a short time, but with both heated specimens the reactions were slight as compared with the reaction to the unheated serum. In one individual, five days after the test, a small localized macular rash appeared around each site of injection. These measured 5.0 cm., 2.5 cm. and 3.5 cm., respectively, in diameter. There was a flat, white, circular area about 1.0 cm. in diameter in the centre of each. The localized rash after persisting for two days disappeared completely. It was apparently a local serum rash.

We attempted to sensitize four guinea-pigs with their homologous serum heated for 120 minutes at 56°C. The initial dose consisted of 1.0 cc. of the serum intraperitoneally. After 36 days, however, none of them showed any definite anaphylactic symptoms when 1 cc. of the heated serum was injected intravenously.

DISCUSSION

The fact that unheated human serum gives rise to practically no reaction when injected intracutaneously into the human skin excludes the possibility that the toxicity of heated human serum is due primarily to the phenomenon of clotting. Peterson (3) reports experi-

ments to show that, immediately after clotting, human serum is toxic for animals, but that 30 minutes to two hours after clotting there is very little febrile reaction following the injection of the serum. We did not use any serum immediately after bleeding, but after standing four hours the unheated serum caused practically no reaction. In using serum for the treatment of hemophilia and other diseases, Widal, Abrami and Brissau (4) found that serum used as soon as possible after bleeding at room temperature produced the minimum of shock. This they thought was due to the fact that there had not been sufficient time for autolysis of the clot to take place. If the serum was allowed to stand (on the clot) at 38°C. for 60 hours, a marked shock followed its injection. There is a possibility that the ability of a serum to produce general reactions runs parallel with its power to produce skin reactions.

Serum drawn from pregnant women, and treated with placental tissue in the cold, but later incubated, produced skin reactions different from those which we obtained. Bronfenbrenner (5) in his experiments found that the reactions appeared more slowly and lasted much longer, even progressing, in one case, to the formation of a sterile pustule.

That heated guinea-pig serum does not cause a reaction in homologous animals seems a remarkable fact, unless we assume that the skin of a guinea-pig will not usually show this type of reaction. Zinsser (6) describes the occasional occurrence of typical immediate skin reactions in anaphylactic guinea-pigs. We were unable to obtain such reactions in a series of guinea-pigs anaphylactically sensitive to horse dander extract. Nor has it been found possible to transfer passively to the skin of rabbits and guinea-pigs hypersensitiveness to the extracts of pollen by the injection of sera from hay-fever patients (Caulfield and Cohen) (7). This could be understood if the skin of these animals was incapable of giving rise to such reactions. Three hypotheses may be advanced to explain the observations that we have recorded: (1) destruction of complement; (2) change in the antiferment-ferment balance; and (3) alteration in colloidal state.

It was at first thought that the destruction of complement might be a factor, but the fact that heating for 120 minutes increased the irritating property of the serum more than heat for 30 minutes, cast

some doubt on this idea. Further, it was demonstrated (Dr. Hyde) that no complement was present in human serum after it had been heated for three minutes at 56°C. We had found that it required 20 minutes' exposure at 56°C. to render the serum capable of giving these reactions. From these results we concluded that the complement content of the serum was not related to its ability to cause these skin reactions.

The second possible explanation involved a change in the antiferment-ferment balance in the serum. In this case we would have to assume that 56°C. was sufficient to destroy the antiferments, but that the ferments would be free to cause proteolysis of the proteins of the serum itself, or the proteins in the tissues about the site of injection. If such products of proteolysis were formed they might produce the reactions.

The hypothesis is untenable according to the work of Yamakawa (8) who found that in guinea-pig serum both the enzyme and the antienzyme were unimpaired after heating at 55°C. for 30 minutes. If the heating were continued, both the enzyme and the inhibiting substance were gradually diminished and were destroyed completely after two hours' exposure to this degree of heat. He was thus unable to dissociate the enzyme from the antienzyme by heat. We have repeated these experiments in a modified form. Four parts of unheated human serum were mixed with one part of heated human serum and the mixtures allowed to stand at room temperature. In four out of six experiments we found that, immediately after mixing the heated and unheated sera, the mixture gave rise to a slight reaction when injected intradermally; after 2½ hours it produced a stronger reaction, and after 21 hours still greater skin reactions. Heated and unheated sera kept at room temperature and mixed just before injection did not cause any greater reaction than a mixture made up of the same sera which had been kept in the ice-chest. In the remaining two experiments the reactions were the same after incubation as before.

The degree of heat necessary to change the antiferment titer of serum was investigated by Jobling and Peterson (9). They reported that if the serum was heated at 54° and 55°C. for fifteen minutes, there was an increase of from 15 per cent to 20 per cent in the anti-

ferment titer. This was especially true if the serum was lipemic. In a number of cases we found the reactions less marked from the serum heated ten or twenty minutes at 56°C. than from unheated serum. In an almost equal number of cases the results were just the reverse. The degree of heat necessary to destroy the antiferment is given as 60°C. for one-half hour by Delezenne (10), but Wells (11) states that 70°C. is required. This latter author also reports that the antiferments are lost by allowing the serum to stand for some time.

In working with guinea-pigs, Jobling and Peterson (12) found that homologous serum may be toxic for animals when the antiferments are removed (*e.g.* treatment with ether), and the serum protease is free to act on the proteins of the serum. If heating the guinea-pig serum at 56°C. removed the antiferments and allowed proteolysis to take place we would expect such heated serum to be toxic for the animals on intravenous injections. This was not the result in five animals. There seems to be no reason to assume that there is any relation between the toxicity of the serum for animals and the production of these skin reactions. Jobling also found (13) that, if the serum was inactivated at 54° for fifteen minutes and then treated with chloroform to remove the antitrypsin, the heated became toxic more slowly than the unheated serum. He explained this by saying that the antitrypsin was removed more slowly from the heated serum. Most of this evidence seems to suggest that heating at 56°C. raises the antiferment titer rather than lowers it.

The most plausible explanation for the irritating property of heated serum would be to assume that there is some alteration in the colloidal state of the serum as a result of heating. This change might take place most rapidly at 56°C., but heating at lower temperatures could quite conceivably cause the same alteration at a slower rate. That 56°C. may cause radical changes in colloidal equilibrium is shown by the fact that fibrinogen present in the plasma is coagulated at this temperature.

DuNoüy (14) found that certain surface tension phenomena which are constant in unheated fresh serum are inhibited by heating the serum at 55° or by allowing it to stand for intervals of several days. This finding strengthens us in the supposition that considerable

alteration in the state of dispersion of the serum colloids occurs when the serum is heated at 55°C.

SUMMARY

1. Unheated homologous human serum causes practically no reaction on intracutaneous injection into human skin.

2. Similar serum heated for thirty minutes or more at 56°C. gives rise to a definite and immediate skin reaction.

3. In most cases the longer the serum is heated at 56° up to two hours—the greater is the reaction.

4. Unheated serum standing at ice-chest temperature for twelve days or more gives similar positive reactions.

5. Heating the serum at lower temperatures for longer periods has a less marked but similar effect on the serum.

6. Heterologous human serum treated in the same way has a similar action.

7. Guinea-pig serum heated at 56°C. for two hours does not cause skin reactions in the guinea-pig's skin.

8. Several hypotheses which might account for the variation in the reactions of heated and unheated sera in human skin are presented. We feel, however, that further investigation is necessary to explain our observations.

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COMBINED ACROCEPHALY AND SYNDACTYLISM OCCURRING IN MOTHER AND DAUGHTER[1]

A Case Report

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I wish to report two cases[2] of acrocephaly in association with syndactylism occurring in members of the same family. Because of the circumstances under which the patients were seen, a complete clinical record is not available and little more can be said than that the deformities of the head, hands, and feet of the mother, aged nineteen years, and her daughter, aged eleven months, were almost identical. I was unable to learn of other members of the family with similar malformations. The photographs must be relied upon to describe and identify the condition.

That acrocephaly and syndactylism occur in combination has been recognized since 1906 when Apert (1) published a report of 8 cases discovered in the literature and of one case from personal observation. In 1920 Park and Powers (2) published an extensive review of the literature on this subject and added several new cases. In this contribution they called attention to several reported cases of oxycephaly and scaphocephaly in which there was a history of familial incidence and cited a case report by Carpenter relating to three children in one family, each the subject of malformations of the head and extremities and also other congenital defects. However, no hereditary factor had been noted in the 29 typical cases of so-called "acrocephalosyndactylism" reviewed by them. J. de Bruin (3), in November, 1925, was able to review 33 cases and says that in none were familial or hereditary characteristics noted.

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[2] The author wishes to express his thanks to Dr. L. R. Poole of Dothan, Alabama for calling these patients to his attention.

The two cases reported here support the view expressed by Park and Powers that the combined malformations are evidences of germ plasm defects rather than the result of externally operating causes.

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Fig. 1. Front View of First Patient (Mother) Showing Malformation of Head, Lowering of the External Canthus of Each Eye with External Strabismus, and Deformity of Hands (Syndactylism)

Fig. 2. Profile of First Patient Showing Abnormal Height of Cranial Vault with Short Antero-posterior Diameter and Exophthalmos

FIG. 3. FEET OF FIRST PATIENT SHOWING WEBBING OF SECOND AND THIRD TOES

FIG. 4. FRONT VIEW OF SECOND PATIENT (DAUGHTER) SHOWING MALFORMATIONS OF HEAD AND HANDS SIMILAR TO THOSE OF HER MOTHER

FIG. 5. PROFILE OF SECOND PATIENT SHOWING TYPE OF CRANIAL VAULT SIMILAR TO THAT OF HER MOTHER

SICKLE-CELL ANAEMIA[1]

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The observations to be reported in this paper were made on six children who were suffering from sickle-cell anaemia of varying severity and on one whose red cells assumed characteristic forms on standing, but who had no anaemia or any other symptom of the disease. In all, fourteen samples of blood were examined by the methods outlined below. I shall not at the present time report the clinical details of these cases, as this paper will deal entirely with the behavior of the red blood cells after they have been removed from the body.

In making preparations for the study of the changes in form of the cells two different methods were used: (1) A small portion of the blood or cell suspension was placed in Hayem's solution and examined in a counting chamber. By this method the proportion of sickle cells could be accurately determined. (2) A fresh coverslip preparation was made, sealed with paraffin, kept at room temperature and examined at intervals up to twenty-four hours or more. This, the usual method, has the disadvantage that the proportion of sickle cells cannot be determined accurately because the tendency of the cells to assume the abnormal forms varies greatly in different parts of the preparation, the variation depending in large part on differences in thickness of the film. On the other hand, this method proved to be of greater usefulness when the proportion of sickle cells by the first method was very small, for in the coverslip preparations the relative number of sickle forms was in general much greater, and in some cases it was only in coverslip preparations that abnormal forms appeared at all.

There was a rather important difference in the appearance of the cells when examined by one or the other of the methods. When the

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first method was used, the abnormal cells had the characteristic crescent or sickle forms seen in the blood of active cases shortly after it is drawn. When the second method was used, the cells might at first have the forms described above, but after 24 hours the appearance was often that of abnormal crenation occurring side by side with normal crenation. In those parts of the preparation where there was no crenation there were usually no abnormal forms whatever.

METHODS OF STUDY

The blood to be examined was obtained by venipuncture and placed immediately in a centrifuge tube containing isotonic sodium oxalate. All glassware used in this work was kept in bichromate cleaning solution until just before use, when it was washed thoroughly in distilled water and drained. The red cells were counted immediately after the blood was drawn. After a time that varied in different experiments, the blood was centrifuged at high speed, and the plasma pipetted off. The cells were then suspended in salt solution (unbuffered) and again centrifuged. This was done six times, and it was considered that the cells were then completely washed. The salt solutions used for washing were saved separately and numbered. After the final washing the cell suspension was made up to a volume that it was estimated would contain about five million cells per cubic millimetre. The cells of this suspension were then counted and the number compared with the number of cells in the fresh blood. By this means it was determined that no destruction of cells had taken place during the washing.

It was noted that during the washing of the cells the sickle forms that were present in the fresh blood after standing had almost entirely disappeared. Since no destruction of cells had taken place during the washing it must be concluded that the sickle cells had resumed their normal shape. Even after twenty-four to forty-eight hours there was no tendency for cells to reassume the crescent forms, and when coverslip preparations were made from the suspension, the cells remained normal in appearance.

The simplest explanation was that some unknown substance which was responsible for the change in the shape of the cells had been

removed from the cells in the course of the washing. If this was so, then the substance, whatever its nature, should be present in the salt solutions used for washing. Accordingly a few drops of the suspension of washed cells were placed in a small amount of each of the six solutions used for washing as well as in salt solution and in the plasma from which the cells had been originally taken. In the case of E. A., on the first examination, there were thirty per cent of sickle cells in the blood after it had been standing for 2 hours. The cells were washed and suspensions made in the washings and in the original plasma as described above. After standing 24 hours the suspensions were examined for sickle cells by the first method. In the solution from the first washing there were practically no sickle forms; in that from the second washing about 10 per cent; in the third about 30 per cent; in the fourth about 10 per cent; in the fifth and sixth none. In the preparation made by replacing the washed cells in the plasma, only about 2 or 3 per cent of sickle cells were present.

This experiment was repeated with the same results in the case of E. A., a little later. In addition, coverslip preparations were made, and after standing 24 hours were examined for sickle cells and photographs made (Figs. 1, 2, 3). Figure 1 shows a twenty-four hour old preparation which was made from the blood as soon as drawn; Figure 2, a preparation of the washed cells replaced in the plasma from which they were taken; Figure 3, a preparation of the washed cells replaced in the third washing.

These results have been abundantly confirmed in other cases. Blood from H. P. was examined five times during an illness in which there were frequent relapses—twice when the hemoglobin was falling rapidly—3 times after transfusions when the condition of the child was stationary. In all instances but one the results detailed above were confirmed. In that one case no sickle cells were seen after twenty-four hours in any preparation. Coverslip preparations were not used in this case. In two other cases results were inconclusive owing to the low percentage of sickle forms. In the four remaining experiments the results obtained in the case of E. A. were confirmed. In all, ten experiments were successful, 3 were failures for reasons that are not at present evident.

In the case of V. M. I found the condition present while examining a number of colored children by making fresh coverslip preparations to be examined several hours later. Her hemoglobin was 100 per cent and her blood at no time contained any characteristic sickle-shaped cells. After standing several hours, however, a coverslip preparation showed changes that were characteristic and that appeared to consist of abnormalities in crenation. Her blood was treated as in

FIG. 1. TWENTY-FOUR-HOUR-OLD COVERSLIP PREPARATION MADE FROM E. A'S BLOOD AS DRAWN

the other cases and coverslip preparations made of the various suspensions. The results confirmed those obtained in the other cases.

Table I shows the results of placing cells from various patients with sickle-cell anaemia and from some with no tendency to form sickle cells in the solutions used for washing the cells from other patients, from two normal children and two with infections, one of them white.

The results are self-evident. In no case did cells from children who did not have the sickle-cell condition assume the sickle forms. Furthermore, as may be seen in the cases of R. S. and V. M., where the number of sickle cells was small or only appeared as abnormal crenation in coverslip preparations, placing the cells in washings from the more severe cases did not cause any increase in the number of abnormal forms. On the other hand, sickle forms appeared abundantly

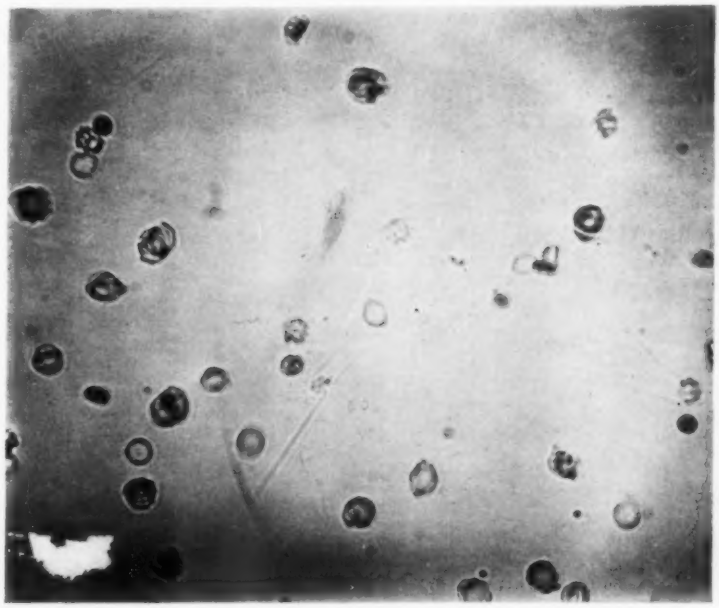

FIG. 2. TWENTY-FOUR-HOUR-OLD COVERSLIP PREPARATION OF CELLS THAT HAD BEEN SEPARATED FROM THE PLASMA, THOROUGHLY WASHED, AND REPLACED IN THE PLASMA

when cells from severe cases were placed in washings from cases in which the blood did not contain sickle cells. From these results, it may be concluded that the substance that becomes attached to the cells causing them to assume the characteristic forms is probably present in all blood, but that only the cells of sickle cell anaemia patients are susceptible to it.

In the earlier experiments it was the third washing in which the greatest proportion of sickle cells were found, but this was not invariable. In one case the only abnormal forms that were present were found in the solution from the first washing; in another case that from the fourth washing was the only one that contained sickle forms. All that I can say about such variability is that I do not yet

FIG. 3. TWENTY-FOUR-HOUR-OLD COVERSLIP PREPARATION OF CELLS THAT HAD BEEN SEPARATED FROM THE PLASMA, THOROUGHLY WASHED, AND REPLACED IN SALT SOLUTION USED FOR WASHING

understand the condition under which the substance is best washed off the cells or re-attached to the cells. An interesting question for study is why the substance remains attached to the cells in plasma but is easily washed off by salt solution. In several cases I separated the cells from the plasma as quickly as possible to determine whether or not an appreciable time elapsed between the drawing of the blood

and the attachment of the unknown substance to the cells. In no case was there any difference in the behavior of the washed cells toward such plasma and toward plasma that had been standing in contact with cells for an hour or more. This result indicates that attachment was either extremely rapid or else had taken place in the blood stream. On the other hand, when the washed cells were replaced in serum instead of plasma, sickle forms appeared.

There are one or two more observations to be recorded: When blood containing sickle cells is centrifuged, the cells become agglu-

TABLE I

CELLS		PERCENTAGE OF SICKLE CELLS IN ORIGINAL BLOOD	WASHINGS							
			Sickle-cell anaemia				Control cases			
			E. A.	H. P.	L. J.	V. M.	Normal (1)	Normal (2)	Typhoid	Pneumonia
Sickle-cell anaemia	E. A.	30	30	–	–	–	20	–	–	25
	H. P.	25	–	25	–	–	–	40	25	–
	R. S.	5	5	–	–	–	–	–	–	–
	L. J.	30	–	30	40	20	–	–	–	–
	V. M.	+	–	–	+	+	–	–	–	–
Normal (1) (colored)		0	0	–	–	–	0	–	–	–
Normal (2) (colored)		0	–	0	–	–	–	0	–	–
Typhoid (white)		0	–	0	–	–	–	–	0	–
Pneumonia (colored)		0	0	–	–	–	–	–	–	0

The figures represent roughly percentages of sickle cells as determined in a counting chamber. In the case of V. M. coverslip preparations were used.

tinated into a mass which, when shaken up in salt solution, is rather stringy and from which a cell suspension can be made only with difficulty. This tendency toward agglutination continues throughout four or five washings and sometimes as many as seven or eight have been necessary to overcome it. In those tubes in which sickle cells reappear, agglutination of a stringy character is also wont to appear. On the other hand, the appearance of this sort of agglutination does not necessarily mean that sickle cells are present, although it usually does. I have tried the effect of solutions of low surface

tension on the formation of sickle cells. The washed cells were placed in tubes containing solutions of sodium glycocholate, sodium oleate, and saponin of varied concentrations. Sickle cells appeared in those tubes in which hemolysis was just beginning. The agglutination phenomenon also occurred in these same tubes.

SUMMARY

These experiments show that when red cells from a patient with sickle-cell anaemia are washed sufficiently, the abnormal cells resume their normal shapes; that when these washed cells are replaced in salt solution used for washing red cells obtained from either normal children or patients with sickle-cell anaemia they reassume the abnormal forms to the same extent as originally, whereas when replaced in plasma they do not reassume the abnormal forms. The simplest explanation of this observation is on the basis of adsorption. An unknown substance common to all blood plasma is adsorbed on the red cells and thereby is removed from the plasma. This substance is capable of changing the shape of a certain proportion of the cells from patients with sickle-cell anaemia. It apparently tends to remain attached to the cells in the presence of plasma, but can be washed off them in salt solution. The cells then regain their normal shapes; but when they are allowed to stand for a sufficient length of time in the salt solution that presumably contains the unknown substance, adsorption again takes place with consequent re-development of the abnormal crescent and sickle forms. What the nature of the substance is that causes the cells to assume the characteristic forms, I do not know. It is probably not any of the constituents of bile. Glycocholic acid was capable of causing susceptible cells to assume sickle shapes but in much higher concentration than is present in blood, even that from patients with a considerable degree of icterus. Moreover, there was no reduction of the surface tension of salt solution after it had been used for washing the cells. The relation of agglutination and possibly haemolysis to the sickle-cell phenomenon deserves further study.

THE PROBABLE PRESENCE OF INCREASED AMOUNTS OF GUANIDINE IN THE BLOOD OF PATIENTS WITH ARTERIAL HYPERTENSION[1]

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During the past two years in a series of communications from this clinic we have called attention to the marked pressor effect of the guanidine compounds when introduced into the body. We have found that suitable amounts of these substances, when introduced intravenously, subcutaneously, intramuscularly or by way of the gastrointestinal tract, produce a definite, marked and prolonged elevation of the blood pressure. These observations have suggested that these compounds may belong to the "spastic components" which some observers have assumed to be present in the blood of certain patients suffering from arterial hypertension.

The observations of Tiegs (1), that sodium nitroprusside in an alkaline solution produces an intense red color with guanidine compounds, has stimulated several observers to employ this reaction for the colorimetric estimation of guanidine. Marston (2, 3) has published a method for the colorimetric estimation of guanidine, and more recently Pfiffner and Myers (4) have applied a modification of it to the estimation of guanidine in the blood and body fluids. Pfiffner and Myers state that guanidine, if present in the normal blood, is probably present in amounts of less than 0.2 mgm. per 100 cc., while in two patients suffering from arterial hypertension color values equivalent to 10 mgm. per 100 cc. of blood were found. These observers emphasize the fact that this color reaction has never been proved specific for guanidine, but it is nevertheless striking that these two patients showed something in large amounts that was either lacking or present in very small amounts in normal blood.

[1] Received for publication November 26, 1926.

We have been working with this color reaction since the appearance of Tiegs' and of Marston's paper, and wish to report some observations with a colorimetric method based upon the Tiegs' work. The discovery by one of us (C. J. W.), that the sensitiveness of the test could be greatly increased by the substitution of potassium ferricyanide for potassium ferrocyanide and that the addition of hydrogen peroxide could be omitted, has stimulated us to apply this color reaction to the study of the blood of patients suffering from arterial hypertension.

Further details regarding this test will be published later, but a few general statements will be made here. With our method we have obtained a color reaction in an aqueous solution as dilute as 1 part of methyl guanidine to 200,000 parts of water. This method in our hands develops approximately twice as much color when applied to control solutions as do those proposed by Marston and by Pfiffner and Myers. Urea, creatinine, and creatine all give some color with the reagent, but the color produced by these three substances is very slight, 0.05 mgm. of methyl guanidine per 5 cc. of water giving as much color as 10 mgm. of urea, 5 mgm. of creatinine, and 0.5 mgm. of creatine. When our method was applied to the study of the blood, it was found that large amounts of added urea produced some color, while creatinine and creatine did not interfere unless very large amounts were added, amounts that are rarely, if ever, encountered even in patients with marked nitrogen retention. When tested by the addition of known amounts of methyl guanidine to the blood, our method showed a recovery of from 75 per cent to 90 per cent of the added methyl guanidine, if amounts of 1 mgm. per 100 cc. or more were added. When amounts less than 1 mgm. per 100 cc. were added, the percentage of recovery was smaller.

METHOD

Reagent.—

Mix

1 volume of sodium nitroprusside	10 per cent
1 volume of potassium ferricyanide	10 per cent
1 volume of sodium hydroxide	10 per cent

Dilute with 4 volumes of water

The solutions of sodium nitroprusside, potassium ferricyanide and sodium hydroxide keep for a long time, but a fresh mixture of the three should be made before carrying out the test. This mixture will keep approximately twenty-four hours.

Method of procedure. Precipitate 10 cc. of oxalated blood according to the Folin-Wu procedure, using hydrochloric acid instead of sulphuric acid. (Any excess of acid will boil off in the subsequent

TABLE I

NUMBER	AGE	BLOOD PRESSURE	"METHYL GUANIDINE"	N.P.N.	UREA	CREATININE	CREATINE
1	16	120/100	0.23	34	12.1	1.2	3.8
2	22	135/80	0.23	33	9.8	1.3	3.7
3	26	120/65	0.23		12	1.7	2.8
4	73	110/65	0.21	28.6	8.9	1.3	3.75
5	43	110/75	0.22	30.6	11.7	1.5	3.65
6	50	110/85	0.22	30	5.1	1.5	4.7
7	18	115/70	0.22	28.6	6.1	1.5	3.25
8	24	118/65	0.20		9.8	1.2	4.8
9	49	134/86	0.31	28.6			
10	55	138/80	0.24	26			
11	34	110/70	0.20				
12	55	125/85	0.20				
13	56	120/54	0.20				
14	35	100/70	0.20				
15	25	134/94	0.25	31.6	14	1.15	3.96
16	21	126/80	0.23	31			
17	31	124/74	0.23	30.8			
18	65	124/76	0.26	31			
19	65	124/70	0.26				
20	46	150/100	0.22	30			

Blood chemistry in milligrams per 100 cc.

evaporation in the case of hydrochloric acid, and neutralization of the excess is not necessary.) Filter and evaporate an aliquot of filtrate (50 cc.) on water-bath to dryness. Extract with absolute alcohol (20 to 30 cc.) and evaporate to dryness. Take up in 5 cc. of water and add 1 cc. of the above reagent. If turbidity develops at this point, the solution may be cleared by filtering through a good grade of filter paper, or better by filtering after the addition of a small amount of barium carbonate. Read against a standard methyl

guanidine solution. It is advisable to make up a series of standards containing 0.02, 0.04, and 0.05 mgm. of methyl guanidine in 5 cc. to which 1 cc. of the reagent is added. We have employed methyl guanidine sulphate as a standard, but have made all of the calculations in terms of methyl guanidine.

TABLE II

NUMBER	AGE	BLOOD PRESSURE	"METHYL GUANIDINE"	N.P.N.	UREA	CREATININE	CREATINE	REMARKS
1	62	210/70	0.48	37.2	16.3	1.2	3.6	
2	49	180/130	0.36		16.3	1.2		
3	30	165/100	0.20		10.3			Very nervous individual
4	50	164/92	0.24	33.4				
5	70	172/76	0.31	41.5				
6	33	218/128	0.70	76				
7	51	172/90	0.30	27.3				
8	63	212/90	0.33	30				
9	65	165/110	0.43	53	33	1.95	2.3	
10	48	220/110	0.42		14.9	1.70	2.6	
11	41	210/148	0.55		25.2	2.2	4	
12	65	265/130	0.89		36	3.2	2.9	
13	54	190/100	0.53		19.2	1.70	2.4	
14	49	170/120	0.53		11.3	1.65	3	
15	65	210/95	0.47		18	1.7	2.5	
16	40	222/148	0.36	30				
17	45	245/176	0.52	78				
		130/60	0.91	86				Cardiac failure
18	41	220/174	0.7	75				
		190/98	0.42	68				
19	35	135/100	0.2	26	7			Chronic nephritis
20	50	140/90	1.03	105	80			Uremia; cardiac failure
21	54	210/144	0.43	36.5				

Blood chemistry in milligrams per 100 cc.

This method was applied first to a study of the blood of a series of patients who had normal blood pressures. Table I shows the results obtained.

The results of this series of observations agree with those of Pfiffner and Myers, that methyl guanidine, if present in the *normal* blood, is in amounts not exceeding 0.2 mgm. per 100 cc. The color produced by 0.2 mgm. of methyl guanidine in the blood is so slight, however, that we cannot be sure whether the test for its presence is positive.

If present, however, in amounts exceeding 0.2 mgm. per 100 cc. it can be detected by this method.

Table II shows the results obtained in a study of 21 patients suffering from arterial hypertension, and in one patient with nephritis who had no elevation of blood pressure.

The table shows that most of these patients showed an increase in what we have termed blood "methyl guanidine." In patients nos. 6, 17, 18 and 20 a fraction of the increased color may have been due to urea, but in the other patients this possibility seems excluded, since they showed normal values for blood urea, creatinine, and creatine, values no higher than those recorded in Table I. While such values as 0.3 or 0.4 mgm. appear to represent very slight increases over the normal figures, yet if the exact value be less than 0.2 mgm. or actually zero, then such amounts are significant.

It is interesting that patient no. 19, who died from nephritis three days after these observations were made, showed no increase in blood "methyl guanidine," and had a normal blood pressure. Several patients with arterial hypertension have shown no increase in blood "methyl guanidine," but as these patients present certain interesting features, they will be reserved for a later report. Patient no. 20 had a marked hypertension before the onset of cardiac failure.

We wish to emphasize that we do not yet know that the substance which gives this increase in color in the blood of hypertensives is methyl guanidine, or some other guanidine compound. These observations suggest, however, that the blood of certain patients suffering from arterial hypertension contains something which is present in greater amounts than in normal blood.

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THE RÔLE OF THE CAPILLARIES AND THEIR ENDOTHELIUM IN THE DISTRIBUTION OF COLLOIDAL CARBON BY THE BLOOD STREAM[1]

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The varied effects of intravascular injections of particulate matter have been studied by many observers for over seven decades. Yet much information regarding the general distribution of the circulating particles in the tissues is still lacking, because most of the investigations have aimed at the illumination of special questions. Cohnheim (1) used this method to determine the origin of pus cells, while Drinker (2, 3, 4) and his associates, as well as Wyssokowitsch (5), Bull (6, 7), Hopkins and Parker (8) and others, have attacked the problems of the rapid cleansing of the blood stream of foreign particles and bacteria. Studies of the reactions of special tissues to circulating granules have been pursued by many, notably by Ponfick (9) and Wislocki (10), who observed the behavior of the spleen and the lung, respectively, from this standpoint.

The only inquiries pertaining to general distribution that I have been able to find are those of Hoffman and Langerhans (1869) (11) and of Nagao in 1920 (12). The earlier authors injected a saline suspension of cinnabar, intravenously, into guinea-pigs. A graded series of doses was given, the animals being killed at various subsequent periods. In brief, they found cinnabar in every tissue examined, excepting the brain and spinal cord; namely, in the lungs, liver, spleen, marrow, kidney, adrenals, probably the gastrointestinal tract, though technical difficulties made them uncertain of this, the cornea, iris, conjunctiva, subcutaneous tissue, striated muscle, but especially that of the heart and tongue, the pia and ependyma, and the lymph nodes. In many of these instances, the particles were found only many days after the injection; the early distribution com-

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prised merely the lungs, liver, spleen, marrow, kidneys, adrenals, and the lymph nodes draining the liver and the site of incision and injection. In these organs the granules were seen either in the capillary lumina, in the so-called endothelial phagocytes of the liver and spleen (not of the marrow) or, in small numbers, in interstitial phagocytes. In the kidneys, the cinnabar appeared mainly in the cortices, and was particularly prominent in the glomeruli. The studies were made with microscopical sections. This work elicited no underlying laws explaining tissue behavior.

Nagao, injecting the unfiltered product of the rubbing of an India ink stick in 8 per cent saline solution, also found that the liver, spleen and marrow took up most of the material immediately after injection, with some aid from the kidneys and adrenals, though at later intervals the connective tissue everywhere revealed carbon-holding phagocytes. His carbon, however, was not in colloidal suspension, and there were inaccuracies in dosage which were said to have been unavoidable.

Apparently the first quantitative work on distribution of particulate or colloidal matter in the tissues was that of Voight (13), who weighed the silver in the tissues of three rabbits, after injecting them intravenously with collargol. The liver and spleen, he found, had taken up most of the material. Sabatini and Salvioli (14), in 1917, learned that colloidal iron, while it may lodge transitionally in the lungs and kidneys, eventually goes almost entirely to the liver and spleen. They noted its presence in Kupffer cells as soon as ten minutes after injection. The quantitative point of view reached its climax in the researches of Drinker, Shaw and Drinker, in 1921 and 1923. Their inquiries into the tissue distribution of colloidal suspensions of manganese dioxide are valuable in themselves and as illustrations of the quantitative method. However, their studies excluded many tissues, and no fundamental principles were deduced from their work. None of these workers has been concerned with the reactions of any tissues except those which are important in the rapid cleansing of the blood stream, or in the storing of the injected material; on the other hand, Ribbert, Bouffard, Goldmann, Kiyono, Aschoff, and others, have made studies of the distribution of colloidal dyes in all the tissues.

Since 1919, most investigators have administered carbon particles in colloidal suspension. In that year, McJunkin (15), injecting

rabbits and dogs with a 5 per cent solution of lampblack in 1 per cent gelatin, made the claim that colloidal carbon particles are taken up solely by the endothelial cells lining the capillaries. Though no precise statement was made, the inference is that the entire capillary endothelium is referred to. Other cells could be seen obviously to contain large numbers of particles; these were mainly the circulating monocytes, and, to a lesser extent, the polymorphonuclear leukocytes. McJunkin drew the conclusion that these monocytes must be derived from the capillary endothelium. Numerous mitoses were seen in carbon-holding endothelial cells, when a solution of sodium citrate containing typhoid bacilli was administered with the carbon, but not otherwise. As Mallory (16) had previously pointed out, "in typhoid fever, the endothelial cells lining the blood vessels proliferate rapidly, and desquamate, or are set free as endothelial leukocytes." Nevertheless, the presence of the mitoses was used as an indication of endothelial proliferative activity, and as support for the idea that carbon-containing endothelial cells normally develop into monocytes. McJunkin summed up his conception in the statement that colloidal carbon particles serve as a specific stain for endothelium.

This article was followed by a series of papers by Foot (17, 18, 19, 20, 21, 22, 23), whose work seemed to be confirmatory of the theory of McJunkin. He used McJunkin's lampblack-gelatin suspension at first, soon changing to India ink, as a colloidal suspension of equal value and much more easily prepared. His ink was diluted with an equal part of distilled water, but was unfiltered. Foot found that carbon particles entered freely the endothelial cells of all the situations that fell under his observation. However, these sites included endothelium only where it lay adjacent to or in an inflammatory lesion produced by interstitial injections of agar or tubercle bacilli, and no normal areas excepting the liver, spleen and lung. None the less, he concluded that McJunkin's idea was essentially correct; that intravenously administered colloidal carbon could be used as a specific label for the generalized capillary endothelium, and that the endothelium was, therefore, the progenitor of all mononuclear cells which contained such carbon; hence, the mononuclear phagocytes in the exudate brought out by agar, and the epithelioid cells of tubercles, as well as the normal blood monocytes, were endothelial descendants. In one

of his later papers (21), however, Foot admitted that no normal endothelium except that of the lung takes up carbon with any degree of activity. In a still later report (22), he was led to the belief that the epithelioid cells in tuberculous meningoencephalitis are exceptional, and do not arise from the local endothelium, but from the circulating monocytes.

In a recent publication McJunkin (24) reported a study of the mononuclear phagocytes of a non-septic peritoneal exudate, by the method of carbon-labelling of the capillary endothelium, now using India ink instead of lamp-black gelatin. Though he believed the mononuclears which contained carbon to be of endothelial origin, he himself was uncertain at the conclusion of the research.

Permar (25, 26, 27), attacking the problem of the origin of the mononuclear phagocytes that can be brought into the pulmonary alveoli by suitable irritation, arrived at the belief that those cells, too, are endothelial products. This conception was attained by the use of the vital stains isamine blue and pyrrhol blue, which, after administration, were found in the endothelium of the lung in small quantity, and again in some of the mononuclear phagocytes of an exudate produced by intra-tracheal instillations of carmine suspensions. His work is referred to at this point because later (28), in a study of the origin of the mononuclear cells of the pneumonic exudate, he employed the carbon-labelling method of McJunkin and Foot, and thereupon was led to make the following statement (p. 43): "Whether the endothelium of the smaller venules and capillaries of the lung alone is active in producing phagocytic cells, or whether the ordinarily more active endothelium of the liver and spleen or other tissues shares or even takes a major part in this activity is a matter about which no dogmatic statement can be made. One cannot rule out extrapulmonary sources. There were certainly many mononuclear phagocytes in the blood stream, as the blood examined in stained films and in lung vessels in the sections clearly showed. It was not possible to say whether or to what extent the monocytes of the general circulation contributed to the mononuclear phagocytes of the exudate. . . . ." All of the cells to which he refers as possible progenitors of the mononuclear phagocytes contained carbon.

Recently, Lang (29), working in Maximow's laboratory, has made

studies of the monocytes in exudates produced about agar in the subcutaneous tissue, after intravenously injecting the rabbits with India ink. He, too, found carbon in large quantities in the local capillary endothelial cells of the lesion. Certain factors, such as the lack of decided changes in the sizes of the endothelial cells, caused him to believe that the particles had not been actively phagocytized by the endothelial cells, but had entered them by phenomena of surface tension. They were thought passively to traverse first these cells, and then the so-called "pericytes of Zimmerman," whence they were taken up anew, and actively, by the local histiocytes. The origin of the carbon-containing mononuclears of the exudate was traced, on a morphological basis, to these histiocytes. The endothelium was definitely excluded, by Lang, from playing any part in the formation of the exudate cells.

Westhues (30) was unable to demonstrate any significant degree of endothelial phagocytosis for Chinese ink or carmine particles in the lung.

The fact, as demonstrated by Foot, Permar and Lang, that endothelium in an inflammatory lesion can take up large amounts of carbon, obviously has no bearing on the behavior of normal endothelium, and is no evidence for the theory that carbon and normal endothelium are specific for one another, though it has been treated as such.

The entire idea of the endothelial origin of free mononuclear cells of any type depends upon the single hypothesis that the endothelial cell is the sole active carbon phagocyte, and its corollary, that the circulating monocytes, which appear in great numbers as particle-holders, are not primarily carbon phagocytes, but are merely budded-off endothelial cells. The truth of this has never been seriously questioned.

The present investigation was made: (I) To attempt to answer the following questions: (A) What is the early distribution in the tissues of circulating particulate material? (B) What are the factors responsible for the distribution, and what are the principles underlying it? (II) To test the primary conception thar carbon is taken up solely and specifically by normal endothelial cells lining the general vascular bed.

There is, in the body, at least one group of cells, the Kupffer cells, which take up carbon particles with great activity, as is generally admitted. If the claim of carbon specificity is to be founded for endothelial cells, it seems fair to demand as part of the evidence the demonstration that they have for carbon a real avidity,—one which is at least comparable to that of the Kupffer cells, and which is unquestionably greater than that of other carbon-holding cells, such as the blood monocytes. Failing such a demonstration, one must be left free to believe that all of the types of carbon-holding cells are equally avid for the granules, and that one group is no more specifically affected than another.

EXPERIMENTAL PART

The experiments were designed to show the early fate of intravenously injected colloidal carbon suspensions, and the comparative responses of the various tissues to the intracapillary presence of the particles.

Materials. The suspensions employed were Higgins' India ink (white-labelled bottle), which is essentially a colloidal suspension of fine carbon particles in acacia, with an admixture of a small amount of camphor; a 5 per cent aqueous suspension of lamp-black in 1 per cent gelatin, prepared according to the directions of McJunkin; and a 5 per cent aqueous suspension of carmine (ground for fifteen minutes in a mortar) in 1 per cent gum arabic. Equal parts of the ink and of distilled water were mixed, and passed through filter paper; the other two substances were neither diluted nor filtered. The tissues studied were those of rabbits of either sex, whose average weight was 2500 grams. The experimental details are presented in Table I.

Technique. Each rabbit, under ether anaesthesia, received one injection of fresh, filtered ink mixture, usually into the right femoral vein, though occasionally the left had to be used, because of accident. The animals were killed by thoracotomy, following etherization. Tissues considered representative of all the types occurring in the body, were removed, and fixed either in Zenker's fluid or in formalin. The Zenker-fixed specimens were cut into sections seven microns thick, and stained with hematoxylin and eosin, or with safranin, while the formalin-fixed ones were either sectioned similarly or, after

being cut into small blocks, averaging one quarter of an inch in thickness, were cleared by the method of Spalteholtz, slightly modified; the blocks, after forty-eight hour fixation, were kept twenty-four hours in each of the solutions in the following order: hydrogen peroxide, 50 per cent alcohol, 95 per cent alcohol, absolute alcohol, benzol, methyl salicylate.

The cleared preparations were used to show the presence or absence of carbon, as well as the comparative particulate content per square area of the various tissues. The stained sections were used for demonstrating the manner of carbon disposition.

TABLE I

MATERIAL	DOSAGE	INTERVAL BETWEEN INJECTION AND DEATH	NUMBER OF ANIMALS
	cc.		
India ink	0.5	1 minute	1
	0.5	1 hour	2
	1.5	1 hour	1
	2.0	1 hour	2
	3.0	1 hour	1
	5.0	1 minute	2
	5.0	1 hour	3
	5.0	24 hours	1
	10.0	4 hours	1
	10.0	48 hours	1
	10.0	120 hours	1
	30.0	4 hours	1
Lamp-black-gelatin	2.0	1 hour	1
	5.0	24 hours	1
Carmine	2.0	1 hour	1
	5.0	1 hour	1

THE EARLY DISTRIBUTION IN THE TISSUES OF CIRCULATING PARTICULATE MATERIAL

1. Results of studies on cleared specimens from rabbits receiving India ink

The surfaces of cleared preparations were not suitable for observation, as they usually bore dust, which could not easily be distinguished from carbon. It was, therefore, necessary to focus beyond the surface,

into the depths of the specimen. The oil-immersion lens, pressed against the surface of the specimen immersed in methyl salicylate, could be used to bring out fine details.

When the cleared blocks, immersed in methyl salicylate, were observed under the low power objective, small numbers of particles could be recognized which were difficult, and sometimes impossible, to identify in ordinary microscopical preparations. Examination of the cleared materials showed that carbon particles are to be found in every type of tissue studied, even after they are no longer circulating in the free state. When compared from the standpoint of carbon content, the tissues clearly fall into the following four groups:

Group 1. Tissues containing great amounts of carbon:
- Spleen and accessory spleens
- Liver
- Marrow
- Lung

Group 2. Tissues containing large amounts of carbon, but not comparable with those of Group 1:
- Adrenal (chiefly reticular zone)
- Kidney cortex.

Group 3. Tissues containing much smaller amounts of carbon:
- Ovary (chiefly perifollicular zones)
- Heart
- Thyroid
- Pancreas
- Small intestine
- Stomach
- Colon
- Diaphragm
- Tongue
- Leptomeninges

Group. 4. Tissues containing extremely small amounts of carbon:
- Kidney medulla
- Abdominal wall muscle
- Gastrocnemius
- Skin
- Adductor magnus
- Brain

The groupings were made by examination of the cleared specimens, in methyl salicylate, under the low power objective. They were

checked by averaging counts of the number of granules in four consecutive low power fields. The counts could not be made with sufficient accuracy to form the basis of a truly precise comparison, but they confirmed regularly the grouping as it had previously been determined. The stomach and colon were, for some reason, well injected in some places and sparsely in others; the lymph nodes, too, were irregularly

TABLE II

The average of ten typical counts from tissues of rabbits receiving India ink

Rabbit numbers: 111, 114, 115, 117, 119, 122, 123, 124, 125, 126

TISSUE	NUMBER OF PARTICLES PER AVERAGE LOW POWER FIELD OF CLEARED SPECIMENS
Spleen	Too dense to count
Liver	Too dense to count
Marrow	Too dense to count
Lung	Too dense to count
Adrenal	149
Kidney cortex	130.5
Ovary	41
Heart	38
Thyroid	37
Pancreas	36
Small intestine	19
Diaphragm	14.1
Tongue	13.4
Kidney medulla	9.6
Colon	8.3
Stomach	5.7
Abdominal wall muscle	3.7
Gastrocnemius	3.5
Skin	3
Adductor magnus	2.8
Brain	1.9

injected, which obviated the possibility of their being accurately counted. Opportunity for counting in the meninges was precluded by their thinness and superficiality, which caused an inevitable confusion of dust and carbon. Further, the counts permitted the arrangement I have given of the tissues within each group.[2] The average of

[2] This arrangement is not absolutely constant, in that there was occasional interchanging of position on the list by two tissues having nearly equal carbon contents; such changes usually depended upon a difference of one or two granules

ten typical counts, from tissues of rabbits receiving India ink, is given in Table II.

The animals receiving lamp-black-gelatin or carmine retained more of the material in the pulmonary tissues than the India ink animals, because those particles became aggregated into larger clumps, *in vivo*. Such clumps were plainly seen in the cleared specimens and in microscopical sections. But the general order and grouping of the tissues was the same in all three series.

In one rabbit (114) an injection of 1.5 cc. of the ink mixture was given into the right femoral artery. The general carbon distribution was identical in this animal with that of those receiving the particles by vein, excepting that the muscles of the right lower extremity retained more of the material than those of the left; most of this excess, however, was in the form of clumps.

In a comparison of the carbon accumulation of the tissues, one significant fact stands out prominently. The order of relative particulate content is constant, regardless of the dosage, and of the interval between injection and death. The single exception is the lung, which, in animals killed within one minute of injection, heads the tissue list, but which rapidly changes to a position below that of the liver and spleen, and probably marrow. The shift is due to its prompt, rapid and continuous loss of carbon. This is concomitant with a gain by the other three tissues, which steadily increase their store.

2. Results of oil-immersion studies on stained sections from rabbits receiving India ink

The particles are in identical situations in all types of tissue. They are found:

A. Within phagocytes, which are free in the capillary lumina, among the red blood corpuscles. Most of these are round mononuclear cells, though occasionally polymorphonuclear ones are seen. Many times such cells are found in small pouches, formed by slight evagination of the capillary wall.

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per field, and were within the limit of error of counting. Rare, striking, unexplained exceptions to the general order were found, as in the myocardium of rabbit 123, which contained almost as much carbon as the adrenal.

B. Within the endothelial cells of the capillary walls. Such cells always retain their positions in the wall, and most frequently their usual spindle shape. Occasionally, however, they are considerably swollen, and, while revealing plainly their attachments to the neighboring cells, project far into the lumen, possibly being on the verge of desquamation. The intracellular particles are always small and discrete. No mitoses were seen.

C. Within the Kupffer cells and splenocytes.

D. Lying upon the endothelial cells, clearly not within the cytoplasm. This appearance is, frequently, just as well defined as that of the granules described in B, which are included within the cell. It is encountered most frequently in the marrow, liver and spleen. In the same tissues, instances are common in which it is impossible to decide whether the particles are outside of or inside the cell.

E. In large, round phagocytes whose position, either in the capillary wall or lumen, can not be determined with certainty. Most of them resemble the circulating phagocytes in size and shape, and are probably intraluminal, but, inasmuch as they touch the wall at some point, they cannot be decisively distinguished from mural cells. This group is most commonly encountered in the lung.

F. Free in the capillary lumina. This appearance is, naturally, most prominent in the animals killed within one minute of injection, and in those receiving the largest doses. Some of the carbon appears in huge clumps in the pulmonary arterioles and capillaries, apparently blocking the lumina, a phenomenon described by Lewis (31) as observed in frogs' lungs, *in vivo*.

G. In mononuclear phagocytes in the interstitial tissue of the organ. These, however, are so exceedingly rare that it may be said the circulating phagocytes and capillary walls and lumina alone are concerned with the early care of the carbon.

H. In the lymphoid nodules between the vessels and bronchi, in the lung parenchyma. This picture is striking after the larger doses, but is seen only infrequently after the smaller ones. No carbon was ever found in unquestioned perivascular lymphatics.

The Kupffer cells give every evidence of being far more active in taking up the carbon particles than the endothelium anywhere, including that of the marrow, liver, spleen and lymph nodes and

adrenals. One hour after a very small dose of India ink (0.5 to 2 cc. of the mixture) and two hours following 2 or 5 cc. of lamp-black suspension, the Kupffer cells contain large stores of carbon granules. Under the same conditions, it is extremely difficult to find in the liver, spleen, lymph nodes or adrenals, endothelial cells which contain any carbon at all; in marrow endothelium one can discover intracellular carbon a little more often. The majority of the endothelial cells there have some relation with small numbers of very delicate granules, which must either coat the cell or lie inside of it; occasionally one can be sure that several particles are enclosed within the cytoplasm. Even the marrow endothelium, however, bears no comparison whatever with the Kupffer cells.

The contrast between Kupffer cells and endothelium is even more strikingly brought out in animals killed within one minute of a 5 cc. administration of the ink and water mixture. Here, the Kupffer cells, in considerable numbers, already contain a quantity of particles, while carbon-holding endothelium, even in the marrow, is rarely to be found. In such livers, most of the carbon-holding cells are quite plainly large Kupffer cells, while a few are found which are difficult to distinguish from ordinary endothelial cells. The great majority of true endothelial cells, however, display no contact with carbon whatever.

Emphasis is placed upon a fact which has been discussed by M. R. Lewis (31), but which has escaped general comment. By no means all the particles seen in relation with an endothelial cell are enclosed within that cell. Many of the particles that stand in relationship with an endothelial cell very definitely lie along the outside of the cell wall. The amount which is actually inside the cytoplasm is usually questionable, and is impossible to determine with accuracy at present.

Endothelial cells bearing no relation of any sort with carbon are occasionally observed in the bone marrow, not only after small doses, but even five days after the administration of 10 cc. of ink mixture, and are common in the liver, spleen, lymph nodes and adrenal cortices under those conditions.

The monocytes, too, display a degree of activity in taking up the carbon, which is fully as great as that of any endothelium, and is probably greater. Carbon-containing monocytes are very numerous, and

they almost always contain more and larger particles than single endothelial cells. In addition, M. R. Lewis (32) reports tissue cultures from chicken lungs and chicken livers, in which endothelial cells and monocytes grew side by side; when colloidal carbon or carmine was added to these cultures, the monocytes engulfed the granules, while large doses had to be given before any particles entered the endothelial cells.

In the remaining organs, phagocytic capillary endothelial cells are relatively few, and widely scattered; they are present in almost every tissue, including the brain, but they are always most numerous where the total amount of carbon is greatest, namely, in the lungs and kidney cortices. They appear, of course, in somewhat greater numbers following the larger doses than after the smaller ones. The renal glomeruli contain carbon-holding phagocytes in fair numbers, but whether they are endothelial or intraluminal, it is impossible to say.

The evidence seems conclusive that, while the endothelium of the marrow, liver, spleen, lymph nodes and adrenals does take up carbon particles, its avidity is not great, and is vastly less than that of Kupffer cells. It may also be concluded that the circulating monocytes display an avidity which is fully as great as that of the endothelium, and is probably somewhat greater.

McJunkin and Foot have laid particular stress upon the pulmonary capillary endothelium as being actively phagocytic for carbon. My own findings lead me to concur with the statements of Wislocki (10), that the difficulty of correctly identifying the phagocytes seen in the pulmonary capillaries is great, and that indubitable endothelial phagocytes are relatively few in the lung.

PERFUSION EXPERIMENTS

In order to test the observation that the bulk of the pulmonary carbon is indeed circulating, rather than fixed, perfusion experiments were performed, as outlined in Table III. The perfusate was warm saline solution. The fluid flowed into the cannulated vessel from a container held three feet above the table.

The perfused lungs from India ink animals clearly supply the information that most of the carbon has been washed out. This fact is

easily demonstrable with the naked eye in untreated specimens, in cleared preparations, and in microscopical sections. The particles remaining in the lungs despite the washing are of three groups: (*a*) Circulating, phagocyted carbon, in small areas still containing blood which had, obvously, escaped adequate washing; (*b*) Apparently immovable clumps, which blocked the vessels; (*c*) Particles within the endothelial cells. The perfusion had washed away the circulating carbon-holding monocytes and the tiny granules on the cell surfaces.

TABLE III

RABBIT	MATERIAL INJECTED INTRAVENOUSLY	DOSAGE	INTERVAL BETWEEN INJECTION AND PERFUSION	PERFUSION
		cc.	*hours*	
130	India ink	5	1	During life, 1 liter into jugular vein; escape from femoral vein. After death, 2 liters through pulmonary artery
140	India Ink	5	1	After death, 1 liter through main pulmonary artery, the right lung being tied off at the hilus
131	India ink	5	1	After death, 2 liters through pulmonary artery
138	Lamp-black-gelatin	2	1	After death, 2 liters through left main pulmonary artery, the right lung being ligated at hilus
139	Lamp-black-gelatin	5	24	Same

In the microscopical sections from such preparations, it is plain that the number of endothelial cells which contain carbon is not great.

The difference between washed and unwashed lungs is less pronounced in the lamp-black-gelatin and the carmine, than in the India ink animals, because of the great numbers of immobile, large clumps in the pulmonary vessels of the former series. However, practically all of the circulating, carbon-holding monocytes and minute particles resting on the cell surfaces are washed away, and microscopical sections show with certainty the moderation of the number of carbon-holding endothelial cells.

It seems fair to consider this evidence conclusive as demonstrating

that the pulmonary capillary endothelium is not avid for carbon particles.

DISCUSSION OF THE FACTORS RESPONSIBLE FOR THE DISTRIBUTION AND THE PRINCIPLES UNDERLYING IT

While the avidity for carbon particles of the endothelium of the marrow, liver, spleen, lymph nodes and adrenals seems feeble when contrasted with that of the Kupffer cells, it is evident that the granules which have entered endothelium are found, mainly in those tissues, and predominantly in the marrow. The nature of this fact is not clear, and it may be, as is generally supposed, that certain moderate differences in degree of phagocytic or adhesive power do exist between the endothelium here and elsewhere.

From the experimental findings, certain mechanical principles suggest themselves as the probable basis of the differences in distribution among the remaining tissues, without assuming differences in phagocytic potency among them. The degree to which these mechanisms apply to the marrow, liver, spleen, lymph nodes and adrenals is uncertain.

It is learned that the material can be found, early and persistently, in the lumina of capillaries of every type of tissue studied, and in considerable quantity in most types. Contrasts may be drawn between the anatomical structures of the tissues at opposite extremes of the distribution list. At the head of the list, excluding the special organs, stand the lungs and kidney cortices; contrasted with these are the kidney medulla, muscle of the abdominal wall, gastrocnemius, adductor magnus, skin and brain.

1. Vascularity of the contrasted tissues. Obviously, where the open capillaries are the most numerous, as in the lung and renal cortex, any given amount of tissue will appear to contain more of a circulating material than one which is less profusely vascularized, such as the brain, or a voluntary muscle. This is a factor which must play an important part in explaining the distribution, in the later stages as well as the early, since the two are identical.

2. Tortuosity of the capillaries of the contrasted tissues. The capillaries of the lung and renal glomeruli are demonstrably very much more tortuous than those of the brain, or striated muscles, whose

channels are particularly straight and long. Circulating particles must be retarded to a greater degree in traversing the tortuous capillary beds than the straight.

3. Relative speeds of the blood flow through the contrasted tissues. It is generally supposed that the blood current is subjected to decided slowing, in the larger capillary networks. However, other factors, such as the size and number of the afferent arteries, the amount of blood reaching the capillaries, and the distance of the part from the heart must combine with the dimensions of the capillary bed, in determining the rate of flow. The problem thus becomes so complicated that it seems impossible at present to make a conclusive comparison of the rates of flow, even in two tissues differing so greatly as resting muscle and the lung. It is probable, none the less, that the stream courses more rapidly through the brain and striated muscles, than through the lungs and renal glomeruli, and that slowing in the latter organs is a factor in determining the carbon accumulation there.

4. The position of the lungs in the circulatory system. The lung is, as Kraus has expressed it, a point of convergence for all the blood, and must receive, per unit of time, an enormously larger dosage of blood and its contents than any other tissue. This fact must play a tremendous part in causing so great a proportion of the carbon to appear within the pulmonary capillaries, and is in itself sufficient to exclude the necessity for special explanations of its particulate accumulation, such as the possession of a sticky capillary lining.

The prompt, progressive and continuous loss of carbon from the kidneys and lungs, as well as from the other non-special tissues, is an additional argument against their possessing any special mechanism for the restraining of particles.

No final statement can be made at present about the vascular condition of the tissues in Group 3. Sections, however, do show that the lung, and at least the glomerular portion of the renal cortex, are grossly much more vascular than are any of the organs of Group 3. It is equally clear that the Group 3 tissues are by no means as sparsely vascularized as are those of Group 4. These considerations appear to explain the intermediate carbon-holding position occupied by these tissues.

The rôle of the endothelial cells

It is evident that the amount of particulate matter found within the endothelial cells in the different tissues is also variable. The renal cortices, and particularly the lungs, display this phenomenon to somewhat better advantage than the other non-special tissues. The non-glomerular portion of the renal cortex is now referred to; in the glomeruli one usually cannot distinguish whether the phagocytes are endothelial or intraluminal. The superiority of these tissues is not so marked, however, as to imply a necessarily greater phagocytic potency, and the appearance appears to be explicable by the following factors:

1. The number of endothelial cells per unit of tissue. The more profuse supply of capillaries in the lungs and kidney cortices than in the muscles, brain, etc., implies a very much larger number of endothelial cells per unit of tissue. The opportunity for contact between particles and endothelial cells and, thus, for phagocytosis, is, therefore, enhanced, and the frequency with which carbon-holding endothelial cells will be encountered in searching a pulmonary or renal field is thus multiplied.

2. Tortuosity. Particles must meet the endothelial walls much more often when flowing through the short and tortuous than through the long and straight channels, thus causing improvement of the opportunity for endothelial phagocytosis.

3. Slowing of the blood current. If the slowing of the blood flow in the wider capillary beds is indeed a fact, it, too, must increase the chance for contact between the endothelium and the particles, and therefore for phagocytosis.

4. The position of the lungs in the circulation. The vastly larger dosage of carbon received, per unit of time, by the lungs than by the other tissues, must bring about immeasurably improved opportunity for contact between endothelium and carbon.

Thus, when we examine cleared sections of representative portions of the body, after the animal has received an injection of India ink, what we see is, essentially, a system of circulating carbon, varying proportions of it being free and in phagocytes. Most of it is seen where the capillaries are most densely crowded. During life, the

particles are, supposedly, passing swiftly and without impediment through some organs, while retardation marks its flow through others. Free particles occasionally enter capillary endothelial cells everywhere, but in the greatest numbers in organs with the most retarded blood or carbon flow. In the liver, spleen, adrenal cortex and marrow, the picture is altered by the particularly great phagocytic potency of the cells there.

The tiny particles found on the surfaces of the endothelial cells everywhere have usually been considered to be adherent to these cells, in a stage just preceding phagocytosis. This conception of them seems to be incorrect. It appears more likely, as Lang infers, that they are merely passively resting against the cells in passing, because of the ease with which they can be washed out of the lung, and because they are found in such tissues as the brain and striated muscles much more easily than are carbon-containing endothelial cells.

CONCLUSION

It is shown that endothelium is not clearly avid for colloidal carbon,—is much less so than the Kupffer cells and is no more so than the circulating monocytes. Therefore, colloidal carbon is not a specific stain for endothelium, and cannot be a test agent of the origin of monocytes from endothelial cells.

SUMMARY

1. A study is reported of the distribution of colloidal carbon and suspended carmine particles in many tissues, from one minute to one hundred and twenty hours after injection.

2. It is found that carbon particles, in small quantities, can be demonstrated within some endothelial cells, especially those of the marrow, liver, spleen, lymph nodes and adrenal cortices; but the amount of carbon which is indubitably within those cells is vastly less than that within the Kupffer cells. Plentiful stores of carbon can be found within the Kupffer cells within one minute of a moderately large injection, but extremely little can be seen within the endothelium, under those conditions.

All the evidence shows that the monocytes are fully as avid for the carbon as are any of the endothelial cells, and the fact that individual

monocytes contain more particles than single endothelial cells suggests that the monocytes are more active.

3. Yet, the endothelium of the marrow, spleen, liver, lymph nodes and possibly the adrenals seems to differ from the remaining endothelium in being able to take up particles in any quantity at all, as is generally understood. The nature of this difference is not clear. Certain mechanical principles are suggested, which appear to account for the differences in distribution among the other tissues. To what degree they apply to the tissues just listed is uncertain. Thus, it is shown that where the capillaries are most numerous, or most tortuous, the carbon appears in greatest quantity. Among the organs not grouped with the reticulo-endothelial system it is demonstrated that the largest numbers of particles are taken into endothelial cells where opportunity is greatest for contact between the endothelium and the particles. Improvements in such opportunity are believed to depend upon the number of endothelial cells per unit of tissue, upon the tortuosity of the capillaries, and upon the dosage of carbon received by the tissue. Fixed differences in endothelial phagocytic power, or in other properties, need not be hypothesized in explaining the differences in the carbon supply of the tissues not classed in the reticulo-endothelial system. The extent to which the same principles can be applied to the latter tissues is not certain.

I wish to express my thanks to Professor MacCallum for helpful suggestions, and to Dr. Rich for his active interest and advice.

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BOOK REVIEWS

Modern Clinical Syphilology, Diagnosis, Treatment, Case Studies. By JOHN H. STOKES, M.D., Professor of Dermatology and Syphilology in the School of Medicine, University of Pennsylvania, with the coöperation of PAUL A. O'LEARY, M.D., WILLIAM GOECKERMAN, M.D., LOREN W. SHAFFER, M.D., and CLEVELAND J. WHITE, M.D. 865 illustrations and text figures. 1144 pages. $12.00. (*W. B. Saunders Company, Philadelphia and London, 1926.*)

This book adds a noteworthy example to the number of monographs in the field of clinical medicine. An intensely personal presentation of the experience of the author and his associates at the Mayo Clinic, it illustrates the value of concentrated study of a single important disease. The author's aim has been to write a text of modern practice in the diagnosis and treatment of syphilis for the student and general practitioner, and to this end he has personally avoided the encyclopedic method of approach. From the clinical standpoint, he devotes the major part of his space to early syphilis, late cutaneous and bone lesions, hepatic and cardio-vascular syphilis, congenital syphilis, and a noteworthy chapter on syphilis of the central nervous system. These are the lesions one sees in everyday practice, and while such rarities as syphilis of the kidney, lung and thyroid are not neglected, they are relegated to their proportional place. The clinical chapters are copiously illustrated, the excellent photographs frequently being given added value by a brief subjoined history of the patient. In addition, there are a large number of what the author calls "thumb-nail sketches"—brief case reports illustrating important points in the text and, from the typographical standpoint, treated as illustrations.

Only 10 of the 22 chapters of the book are devoted to the purely clinical description of syphilis. The remaining 12 chapters consider those very questions which so often perplex the student, practitioner, or teacher; and their subject matter is presented in such a way as to make the book of the utmost practical value. The discussion opens with a chapter on the fundamental bacteriology, pathology and immunology of syphilis. The keynote is here sounded by the author's parable of the seed, the soil, the weather, the cultivator and the crop. The seed is the Spirocheta pallida, the soil is the host, the cultivator the physician who controls the weather of treatment. The interaction of these elements produce the ultimate outcome of the disease, the crop. This concept is carried in mind throughout the whole book, much to the advantage of visualizing syphilis from the dynamic standpoint.

Excellent chapters on the clinical approach to syphilis, the fundamental diagnostic tests, and the fundamental principles of treatment follow. Here one will miss the customary (and futile) technical description of the Wassermann test, finding

instead splendid paragraphs on the interpretation of the test as applied to blood and spinal fluid. The chapter on principles of treatment should be studied and restudied by all those accepting the responsibility of syphilotherapy.

Next come two chapters dealing with the weapons of treatment—mercury, bismuth, the iodides, and the arsphenamins; and then two chapters providing minute descriptions of the technique of treatment.

Drug reactions, complications and contraindications are considered in a separate chapter. Perhaps the most important of the treatment chapters, however, are those devoted to collateral therapeutic factors, and to the management of early syphilis. In the former are considered in detail such important questions as when to treat, how much to treat, infectiousness, hygiene, the response of syphilis to therapeutic effort (with a noteworthy table providing the author's experience as to the percentage improvement and the ultimate therapeutic result to be expected in all types of syphilis), serologic control of treatment, the therapeutic test, and other miscellaneous topics. In the latter is given a summary of the best current practice in the treatment of early syphilis. The management of late, cardiovascular, hepatic, central nervous system, and congenital syphilis finds due consideration in the clinical chapters dealing with these subjects.

In general, the book is so good that it is difficult to find fault. The most striking omission is that of a critical bibliography, which the author explains was an unavoidable consequence of the limitation of the work to a single volume. Though the book supplies a long felt need by assembling under a single cover information heretofore available only by painstaking study of the current literature, the absence of references is deeply to be regretted. In subsequent editions it may perhaps be possible to supply a bibliography, for which space might be gained by the omission of an unessential chapter of 21 pages on the physical examination for syphilis, the gist of which could easily be condensed to one paragraph without illustrations, and by the considerable shortening of the 88 pages devoted to a description of the minutiae of venipuncture, arsphenamin administration, spinal puncture, etc. The material of these three chapters can be readily found in many other books; the remaining 20 chapters form a record of expert personal opinion and experience such as is nowhere else available.

The author's style is refreshing, many of the case records, for example, being written in the vernacular, and makes for easy and delightful reading.

In summary, the book is one which is likely to prove of the utmost value to students, and to all physicians whose daily work brings them into contact with syphilis—which is to say, all practicing physicians. It is hardly too much to say that it is quite the best modern presentation of the subject.

J. E. M.

Local Anesthesia in Otolaryngology and Rhinology. By JAMES J. KING. 21 illustrations. 205 pp. $5.00. (*Paul B. Hoeber, Inc., New York City, 1926.*)

This book offers a very good description of the use of local and regional anesthesia in otolaryngology and rhinology. Although the methods described do not

vary in their main features from those found in many other works, the presentation and discussion by one who has had extensive experience in the application of standard methods to a special field is always valuable. Those interested in the history of the development of regional anesthesia will find a comprehensive outline of its chronology.

The second part of the book is of particular interest. It deals with the broader aspects of the entire subject of regional anesthesia and includes the report of the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association. This report is based on the results of a questionnaire to which eighteen hundred replies were received. The tabulated results furnish considerable formation on the fatalities and untoward results occasionally encountered.

The book is, in short, useful not only in its relation to otolaryngology, but also for this report, the study of which will be profitable to all surgeons interested in local anesthesia.

E. M. H.

The Human Cerebrospinal Fluid. An Investigation of the Most Recent Advances, as Reported by the Association for Research in Nervous and Mental Disease. xxiii plus 568 pp. 75 illustrations and 58 tables. $10.00. (*Paul B. Hoeber, Inc. New York, 1926.*)

In 1924, the yearly meeting of the Association for Research in Nervous and Mental Disease was devoted to a consideration of the human cerebrospinal fluid. This volume contains the material presented at that meeting and includes a section on the normal cerebrospinal fluid of man.

The clinical reports in the volume are based upon more than five thousand examinations of the cerebrospinal fluid obtained from almost all the known and conceivable pathological states. Each chapter contains a review of the pertinent knowledge of the past on the phase of the subject with which it deals, a presentation of the author's investigations and a bibliography. These data are well presented and are accessible through a good index. Throughout the book there is a sane emphasis on the thorough neurological examination of a patient whose liquor cerebrospinalis is to be submitted to the fateful dictum of the laboratory. Important chapters in the book, broader in conception than a compilation of its characteristics, deal with the formation and absorption of the cerebrospinal fluid, changes in brain bulk through the use of salt solutions given intravenously, procedures for obtaining fluid from ventricles, cistern and spinal sac, manometric studies, and roentgenography of the central nervous system through the use of injections of air and lipiodol. The volume concludes with resports of studies on the treatment of diseases of the nervous system by way of the cerebrospinal fluid, with a chapter by the late Dr. Fordyce on the intraspinal treatment of syphilis of the central nervous system.

The thirty-nine contributors to this volume comprise many men whose minds are not always in accord on the same subject. The divergences of opinion, however, are astonishingly few, although they concern, for example, such structures as the Pacchionian granulations in man. There is also a gradation in the merit of the contributions. It seems unnecessary to space two paragraphs on page 43 with separate captions for "cholesterin" and "cholesterol," and the language on page 77, where a depot of spirochetes is said to be surrounded by an "admiring circle of cells," verges on the picturesque through a typographical error. On page 326, the criteria given as necessary for establishing a distinction between the typhoid and paratyphoid bacillus suggest that some of the authors may have written about things with which they were not entirely familiar.

These slight errors, however, do not mar the usefulness of this volume. It fulfills the intentions of the Association which sponsored it, as a book containing not only a record of present-day research but capable also of serving as a standard reference book on the human cerebrospinal fluid.

S. B-J.

Osler's Modern Medicine, Third Edition, re-edited by Thomas McCrae, M.D., assisted by Elmer H. Funk, M.D. Vol. III. Diseases of Metabolism—Diseases of the Digestive System. 1052 pp. $9.00. (*Lea and Febiger, Philadelphia, 1926.*)

The articles are in the main by the same authors as those in the previous edition, and the same general tone is maintained. One exception is that the chapter on the liver and biliary tract is by Lyon, and is colored by the scheme of diagnosis and therapy of which he is the exponent. In speaking of non-surgical drainage of the biliary tract, he says that "this method has been generally accepted as a practical procedure, both diagnostically and therapeutically."

The most interesting chapter is DuBois' article on "General Considerations of Metabolism." It gives in attractive form a brief sketch of various phases of metabolism of every day application, kept free from details too technical for the general reader.

This continues to be a comprehensive, useful work to which the busy doctor can turn for review and for help with his individual patients. It answers many of the questions which constantly come up before the practitioner whose bedside experience raises queries which must be answered readily or passed over until another day.

J. L. D.

The Abdomen in Labor. By NORMAN PORRITT. 73 pp. $1.75. (*Oxford Medical Publications, Humphrey Milford, London, 1926.*)

This little book, written by a general practitioner of thirty-eight years' experience, stresses the importance of careful abdominal examination in every labor. He shows with "touch pictures" how the diagnosis of presentation and position can be made and how one can follow the descent of the presenting part without the

use of vaginal examination, thereby lessening the chances of introducing bacteria into the birth canal. A strong plea is made for the adoption of the dorsal position during labor instead of the left lateral position so universally employed among the English.

J. K. H.

The Stomach and Upper Alimentary Canal in Health and Disease. By T. Izod Bennett, M.D. (Lond.) M.R.C.P. 154 pp. $3.25. (*Humphrey Milford, Oxford University Press, 1926.*)

In this small and in many ways commendable book, the author follows the usual routine, presenting first the physiology of the mouth, and following this by a study of diseases of the tongue, buccal mucosa, salivary glands, teeth, gums, pharynx, and tonsils. Several illustrations showing dental radiograms depicting pyorrhea alveolaris and root abscess are excellent, and should be helpful in demonstrating the value of this method in diagnosis. The section devoted to the esophagus is well written, in fairly full detail, and gives recent ideas regarding methods of diagnosis and treatment. The next 192 pages are given to the stomach,—its physiology and diseases,—the former being quite comprehensively dealt with and brought up to date. The chapter closes with a fairly adequate bibliography.

In developing his thesis upon the examination of patients, the author emphasizes the value of the fractional method of gastric analysis in diagnosis of gastric disease —a view not shared by many workers in this country.

When dealing with the chemical examination of feces, he aptly directs attention to the slight value of a single negative test for occult blood, correctly stressing the need for a series of such tests on successive days, so that the presence or absence of a bleeding lesion may be conclusively established.

The chapters on peptic ulcer, including pathology, symptomatology, complications and treatment, are well presented, being concise though comprehensive. He calls attention to the term "juxta-pyloric," adopted—and justifiably so—by the Continental writers for the classification of ulcers occurring in the pyloric canal and duodenal bulb, in preference to the terms "duodenal" and "gastric," according as the ulcer is proximal to or distal from the pyloric vein.

A chapter of negligible value is devoted to the duodenum, pancreas, and liver.

All things considered, the author has made a very creditable presentation; and although from its very smallness its value must inevitably be lessened by the lack of many important details, particularly about the pancreas and liver, the book may certainly be recommended to practitioners, if not to students, for quick and ready reference in the solution of salient questions.

E. H. G.

Practical Materia Medica and Prescription Writing. By Oscar W. Bethea, M.D., Ph.G., F.C.S., Professor of Therapeutics, Tulane School of Medicine. 498 pp. $4.50. (*F. A. Davis Co., Philadelphia, 1926.*)

The book is divided into three parts. The first treats of official and unofficial drugs, their therapeutic action and uses, alphabetically arranged; part two con-

tains instructions for the proper writing of prescriptions and part three shows actual examples of correct and incorrect forms.

In part one the general procedure employed for the study of each drug consists of the official and unofficial names and synonyms along with the official definition of the medicinal agent. This is followed by a list of the various preparations of the drug, a discussion of their therapeutic activity and a statement of their uses and manner of administration. Of special interest to the clinician is the fact that after the discussion of the most important drugs, there are recorded several prescriptions typical of those in which the drug or its galenic preparations occur.

The second part of the treatise is introduced by a thorough discussion of metrology and the use of the various systems of weight and measure in physician's prescriptions. All parts of the prescription are carefully discussed and illustrated freely by examples. The author particularly emphasizes the use of correct prescription Latin by physicians and urges coöperation between the compounder and the prescriber, namely, the pharmacist and the physician.

The examples of prescriptions exhibited in part three are so well discussed in regard to correctness of Latin, incompatibility and error in dosage that the medical man may become familiar with the usual errors and thus endeavor to avoid them.

On the whole, this book is one that is admirably suited for a text in the teaching of this particular phase of pharmacology to the student of medicine.

J. C. K., Jr.

The Heart. By Alexander George Gibson, D.M., F.R.C.P. (Lond.), Physician to the Radcliffe Infirmary. 108 pp. $1.50. (*Oxford University Press, 1926.*)

In response to the popular demand for quick and easy roads to knowledge we now have in pocket size a book purporting to cover the entire field of heart disease in 108 pages. Necessarily, such a volume can be nothing more than the merest outline, of no interest whatsoever to the medical student or practitioner for whom unfortunately this field of medicine has not as yet become so agreeably simplified. Being a mere outline, no space is left for the qualifying statements which cluster so confusingly about the phrases of more cautious writers. The result is a very readable little book presenting in non-technical language the outstanding features of heart disease as understood by the Mackenzie school. It should be of real interest to nurses and social workers.

J. M. F.

PROBLEMS OF THE RELATION OF MUSCLE AND NERVE[1]

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It has always seemed a very interesting phenomenon that the advance of scientific knowledge is not an orderly, regular process but rather a series of slow steps, gropingly attempted, with an occasional swift rush of epochal achievement. All great endowments and universities place their faiths upon the slow steps, upon the gradual assembling of the materials out of which will ultimately be built the rambling manor-house of scientific knowledge. The attitude of administrative mind which affords facilities for this tedious and difficult accumulation of new data, for the reassembling of the old, is unquestionably sound; genius is unhampered in this system and may use to advantage the facts gathered by those of lesser intellectual attainments.

But one may with justice question the accuracy of this picture of the orderly accumulation of scientific fact. Is it really true that the essential body of biological data is built up by the laying of a number of new foundation-blocks in regular intervals, or is it not closer to the truth to assert that many of these foundation-stones are soon to be hurled aside as imperfect or false, as additional knowledge, derived by more adequate control or by employment of more accurate methods, becomes available? These questions intrigue thought: as the topic which I am to discuss is studied, it becomes apparent that in many regards new knowledge has been added to the old, only to discover that the old conception is incorrect or at best inadequate. Because of such discrepancies, the problem of the relation of nerve and striated muscle is today one with which many workers are concerned and one which promises much in its solution for the advance of anatomical and physiological learning.

[1] Address delivered at the formal exercises to mark the opening of the School of Medicine and Dentistry, University of Rochester, Rochester, N. Y., on October 25–26, 1926.

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The control of voluntary muscle movement by the central nervous system through the agency of the peripheral nerves is an element of physiology dating back to the early epochs of medical knowledge. The importance of this relationship was recognized by Galen, who in the second century hypothesized an outflow of animal spirits from the central nervous system along the peripheral nerves to each individual muscle. This well known conception controlled medical thought for many centuries; like many others of Galen's conceptions it had in it the suggestion of modern knowledge of the nerve impulse in its activation of muscle contraction.

During the last five or six hundred years, anatomists, unsatisfied with information restricted to the gross connections of main nerve trunks with the voluntary muscles of the body, investigated the finer terminations of the large nerves, finding that each nerve divided within the muscle mass into smaller branches which possessed definite union, in some way quite unknown, with the muscle fibers. But it was not until a century ago, that new knowledge, amplifying the old, was had. First, and most important, came the discovery by two great investigators, Charles Bell and François Magendie, that the anterior roots of the spinal nerves are motor in function; the posterior, sensory. And soon thereafter, with the development of the microscopic study of the animal body, histologists demonstrated that each peripheral nerve is composed of individual minute fibers and similarly each gross muscle of individual fibers. Search was inaugurated for the terminations of these individual nerve fibers upon the individual muscle fibers; the result of this search was initially a suggestion,—or, most charitably, a mistaken observation,—that each individual nerve fiber formed a fine noose-like loop about the individual muscle fiber, thus affording a means of transmission of the "nerve impulse" into the muscle.

This conception of a loop-like termination for the nerve fiber held until histologists were able to see actually the mode of ending of nerve upon muscle. Of these modern studies, the initial step was taken by Doyère (1840), who demonstrated that the medullated nerve fiber entered into a distinct, highly nucleated elevation on the muscle fiber: Doyère's hillock still forms an essential factor in present day descriptions of the histology of nerve termination. Then in the early part of

the eighteen seventies, knowledge was markedly advanced by the splendid studies of Kühne (1862) and Engelmann (1863), who were able to follow each medullated nerve fiber into a terminal network, lying under and within Doyère's eminence, and constituting an arborization of nerve tissue intimately connected with the muscle fiber itself.

These observations, verified soon by many workers using different methods of demonstration, afforded then the first foundation-stone in our present-day knowledge of the anatomical relationship of muscle and nerve. The stone seemed definite and well laid—the anatomy necessary for the conveyance of nerve impulses from the central nervous system by way of anterior root fibers under the Bell-Magendie law to voluntary muscle was established. The subject as far as further scientific data were concerned appeared complete and finished. Such other observations as were made during the next two decades seemed merely to furnish refinements without giving fundamental additions to our knowledge of this relationship of muscle and nerve.

In one regard, however, information regarding the mode of termination of medullated motor nerves was soon considered inadequate. Largely due to the researches of physiologists, there was brought forward functional evidence that there exists some substance, tissue, or protoplasm between the arborized termination of the nerve and the muscle fiber. The histological search for this substance, unstainable by present day methods, has remained fruitless, even though some investigators, notably Sutton and Boeke, have closely approached its solution. But with this exception, the primary relationship of medullated motor nerve to voluntary muscle was established in a very complete sense fifty years ago.

Accepting these observations as fundamental, it must have seemed quite futile for investigators in the final years of the last century to attempt any further studies of the nerve-muscle complex. Yet Sherrington (1894) undertook to determine the component fibers in peripheral nerves, selecting particularly the nerve to the vastus medialis muscle in the cat and monkey. Sherrington soon ascertained that approximately fifty per cent of the fibers in such a muscle-nerve were sensory and that these sensory fibers, largely covered with a myelin sheath, had their peripheral terminations in the so-called muscle-

spindles. These interesting structures, in mammals composed essentially of a central core of striated muscle fibers lying in a fluid space within a laminated capsule, had already been described by histologists, without definite suggestion as to their functional significance, though Onanoff (1890) antedated Sherrington's demonstration of their sensory rôle by a few years. Weismann (1860), to whom credit for the initial discovery of the spindle is given, merely identified the intrafusal bundle of highly staining muscle-fibers. Soon thereafter Kölliker (1863) described similar collections of striated fibers in adult amphibian muscle and ventured the hypothesis that these constituted a center for embryonic growth; and other workers advanced still other ideas as to their significance. Sherrington's demonstration that these muscle-spindles constituted a sensory organ for voluntary muscle was unequivocal and definite; his experiments, based on degeneration of the motor fibers from the anterior roots, with the sensory fibers intact though isolated from the central nervous system, seemed to furnish the last link in a completed story of the relationship of muscle and nerve. The mode of ending of the sensory nerves on the intrafusal fibers had been seen by Kerschner in 1888; Kölliker's (1889) and Ruffini's (1892) studies of the subject supplied the final elaboration of the relationship. More recent work on the non-medullated innervation of the intrafusal fibers of the spindle, incomplete as it is today, has not affected at all the concept of the sensory mechanism of the spindle.

With Sherrington's publication another chapter in our general topic was apparently completed and on it was quickly built a comprehensive hypothesis of muscle tone and muscle sense. It was possible then to explain the early fundamental observations of Brondgeest, unfortunately lost for forty years. For in 1860, this Dutch investigator had conclusively shown that the tonus of the hind-leg of the frog was destroyed by section of the posterior roots of the nerves leading to the muscles concerned. Sherrington, pursuing his important studies, was able in the course of the next twenty years (1894–1915) to construct an epochal theory of muscle tonus as the postural reaction of the organism—a theory which today properly dominates physiological thought. The experimental basis for this hypothesis was largely the study of the reactions of the decerebrate animal. In this preparation, following transection of the brain-stem in the region of the

colliculi, the extensor muscles—those resisting the pull of gravity—exhibit a prolonged contraction enduring for many hours. This extensor spasm was shown by Sherrington to be dependent upon the inflow of impulses from the muscles themselves and to be mediated through the extra-pyramidal motor tracts of the central nervous system. Thus again appeared a fairly adequate anatomical and physiological study of the important subject of tonus—the initial sensory mechanism being the muscle-spindles lying in the muscles themselves, inflow of impulses along sensory nerves to the central nervous system, integration within centers in the midbrain, caudal conduction by extra-pyramidal fibers and final outflow by medullated motor nerves to the end-plates on the muscle-fibers.

During the period of Sherrington's active studies, another series of researches were being conducted by histologists which, almost unnoted at first, have in the last few years come prominently to the forefront of anatomico-physiological investigation. These observations really date from the initial discoveries by Tschiriew (1879) and by Bremer (1882) that non-medullated nerve terminals occur on striated muscle. Considered to be merely non-medullated branches of medullated motor nerves or to be embryonic forms, these structures were not for many years held to have any essential relation to muscle and nerve. Twenty years later Perroncito (1902) gave an additional stimulus to the study when he described both medullated and non-medullated motor fibers terminating on voluntary muscle fibers: but whether these unmyelinated forms were merely branches from vascular plexuses or were ultra-terminal endings of the regular myelinated fibers was not determined. Perroncito, however, ventured the suggestion that the medullated and non-medullated fibers actually end on different kinds of muscle-fibers—the small granular and the large non-granular. The existence of two types of muscle-fibers, the red and the white, had previously been effectively demonstrated in 1887 by Ranvier, whose observations were soon confirmed and extended by Grützner (1887). Almost at once the difficulty of homologizing the red and white types with the granular and non-granular forms arose; the problem was attacked by anatomical, chemical and physiological methods. Here then was the field set for hypothesis and deduction: in addition to the confusion arising from two types of striated muscle-

fiber, the incomplete anatomical observations on non-medullated motor branches to voluntary muscles were sufficient only for speculation; and it is a very fortunate thing that the next important steps in the development of our concepts were taken by an anatomist.

It is to the credit of Boeke that we now have very definite knowledge that voluntary muscles possess anatomically both medullated and non-medullated motor innervations. Beginning his publications in 1909, Boeke first confirmed the terminations of non-medullated fibers in grape-like forms, as described by Tschiriew, and established their hypolemmal position. Boeke held strongly to the view that these non-medullated fibers were not ultraterminal, but by his purely morphological studies neither he nor Botezat (1910), who identified similar structures in the voluntary muscles of the bird, were able to establish their central origin. Later Boeke, both individually and in association with other workers, succeeded, by means of critically devised degeneration experiments, in demonstrating that voluntary muscles are doubly innervated through medullated voluntary fibers and non-medullated efferent sympathetic fibers having a hypolemmal termination. Agduhr (1919) similarly identified a hypolemmal ending for most of the non-medullated fibers in the arm musculature, but found also some non-medullated terminals in an epilemmal position.

The uniformity of the findings of these workers indicates in definite fashion the existence of the two sets of terminals on individual striated muscle-fibers. It must be realized, however, that the observations of Boeke and his associates have all been based on a single histological method—that of silver impregnation—and the suggestion has been put forward, particularly by English writers, that these non-medullated fibers might well be finely myelinated fibers, whose sheaths resisting total or partial degeneration, had become impregnated with the silver. It is also obvious that even in carefully planned somatic degeneration experiments, the non-medullated nerves of the vascular system will resist degeneration and that therefore a plexus of non-medullated nerves will unquestionably be found in the subsequent histological preparations.

Following the impetus given to these studies by Boeke's findings and their immediate application to physiological thought, other anatomists undertook investigations of this morphological relationship of

non-medullated nerve fibers to muscle fibers. Notable among the researches based on purely histological methods are the reports of Kulchitsky (1924) on the frog and python. This investigator, in his paper on the python, pictured definite terminations of non-medullated nerves on the small-sized striated fiber and the well-known motor end-plate type of termination on the large muscle-fiber. Unfortunately, Kulchitsky's preparations were studied by other observers and different reports of diverse findings are at hand. Hunter, (1924) largely on the basis of Kulchitsky's work and on certain poorly controlled observations of Latham (1925), was led to the belief that the large muscle-fibers were innervated by medullated somatic nerves and the small fibers by non-medullated sympathetic nerves. Garven's (1925) recent studies, from material impregnated with gold chloride, contradict the assumption of Hunter and indicate that both the large and small muscle-fibers may be innervated by branches of the same medullated nerve. Today, however, this essential phase of the subject bids fair to be untangled by the studies of Marion Hines (1926). In a preliminary report last spring, Hines pointed out that, in vital methylene blue preparations of the frog, the skeletal muscles contain both large and small fibers, and that often a single axis cylinder of a medullated nerve innervates both types of fibers. In some instances, Hines traced a non-medullated nerve fiber into the typical motor end-plate of a medullated nerve; by degeneration experiments, these unmyelinated fibers were proved to be sympathetic. The extension of Hines' studies to mammalian muscle will do much in establishing conceptions of the morphological relation of non-medullated nerves to striated muscle.

Coincidentally with the development of anatomical knowledge of the relationship of the sympathetic system to voluntary muscles during the past twenty years, has come marked physiological interest in this subject. The first of the suggestions regarding a functional importance of the non-medullated fibers running to striated muscle followed closely upon Perroncito's descriptions and was advanced by Mosso (1904), who held that the non-medullated fibers were sympathetic in origin and governed tonus and slow contraction, while the cerebrospinal or voluntary fibers were concerned only with quick clonic movements. Some years later, de Boer (1913), having Boeke's mor-

phologic evidence that the non-medullated fibers were sympathetic in origin, extirpated the lumbar rami communicantes in a frog and observed a subsequent ipsilateral loss of tone. De Boer, in consequence, interpreted his findings as establishing the sympathetic control of skeletal muscle tonus.

Since the publication of de Boer's first paper on this subject in 1913, many investigators have studied this relationship of the sympathetic system to muscle tone. The greatest diversities of results from apparently similar experiments, performed under apparently similar conditions, have been reported, and interested observers have difficulty in ascertaining the basic truths in the subject. Largely due to the enthusiastic championship of John I. Hunter (1924), the suggestions of Langelaan that there are two elements in tone—a contractile tone and a plastic tone—have been brought prominently before the physiologists. While the general subject of tonus cannot be presented within the limits of the present paper, a few comments may not be amiss as the subject is so closely bound up with our general topic.

At the present time the weight of evidence, both experimental and clinical, is unquestionably opposed to the conception that the sympathetic system is responsible for the maintenance of tone in skeletal muscle. The reasons for expressing disbelief in the idea, which is so attractive and would fain be accepted by most workers, are many; perhaps the first of these reasons is that direct stimulation of the sympathetic nervous system has failed in the hands of most observers to increase existing tonus. In general, also, excitation of this system by drugs capable of stimulating the endings of the non-medullated sympathetic nerves has not resulted in augmentation of tone. The positive results,—loss of tone after extirpation of the sympathetic elements—as reported initially by de Boer and subsequently in modified forms by other workers, are all subject to other interpretations. The cutting or extirpation of the sympathetic fibers to one leg of an animal does not merely remove one set of non-medullated fibers—as for instance, those running to skeletal muscle and possibly concerned with tone—but it removes all kinds of sympathetic fibers,—as for instance, those concerned with vasomotor control. Examined critically, it is wholly possible to consider all of these positive results, so far obtained, as being due to the removal of vasomotor or other influences rather than

to the removal of the fibers possessing direct control over the tonic contraction of muscle.

During the past year, however, one series of observations has been published which seems to afford more indisputable evidence regarding this whole subject than we have had before. These are the experiments of Coman (1926), who after obtaining no indication of sympathetic control by means of electrical stimulation or extirpation of the sympathetic, devised the crucial experiment of removing all of the voluntary cerebrospinal fibers to the fore-leg of two different laboratory mammals and leaving the sympathetic fibers intact. Coman accomplished this experimental set-up by intradural section of the anterior roots of the cervical nerves forming the brachial plexus, in this way not interfering with the thoracic sympathetic outflow destined for the fore-leg. In Coman's acute experiments, decerebration followed closely upon the experimental nerve section: under no condition was there evidence of any tonus in the operated leg. Similarly, if the section of the anterior roots was made under sterile precautions and the animal then allowed to recover, no tonus was exhibited in the affected leg: in every case, the leg remained absolutely flaccid, exhibiting neither plastic nor contractile tone. When decerebrated after intervals of several days up to two weeks, these animals showed a normal outspoken rigidity in three legs while the leg deprived of cerebrospinal fibers remained absolutely flaccid. Coman's experimental procedure, preserving as it does all sympathetic fibers to the fore-leg and preserving also all sensory fibers so that the necessary inflow of proprioceptive influences could be effected, seems to yield the most conclusive evidence thus far at hand; his negative results indicate that the sympathetic system is at least not directly concerned with the maintenance of tone of skeletal muscle.

Even such irrefutable negative findings as those reported by Coman do not in any way solve the problems connected with the termination of non-medullated sympathetic fibers upon skeletal muscle. Sherrington's demonstration of the function of muscle-spindles, taken in conjunction with Brondgeest's experiments, seemed to have given all of the necessary physiological data for the sensory proprioceptive factor in tonus, the final impulses traveling by way of medullated nerve-fibers to the motor end-plates. Thus was had an adequate theory of

tonus, based on well substantiated morphology and on well-controlled physiological experimentation. With the advance of anatomical knowledge, particularly with the description of non-medullated sympathetic nerve-fibers terminating on striated muscle, our well established theory seemed at first to yield to newer physiological findings when reports regarding the loss of tonus following sympathectomies were published. It now appears, however, that these non-medullated fibers are not directly concerned with the tonic contraction of voluntary muscle, and today there is opened up a very fascinating field of combined physiological and anatomical investigation regarding the function of these fibers. Is it remotely possible, as suggested by Orbeli's (1923) work, that these are trophic fibers concerned with the metabolic processes of muscle, when the preponderance of data is opposed to any conception of a separate set of nerve-fibers having to do with nutrition? Or is it possible that these sympathetic fibers have some well-hidden function in initiating the contraction of the muscle? Or do they act merely as reinforcing or substantiating fibers making more effective the cerebrospinal nerves in their control of muscular contraction? These are a few of the functional possibilities which come immediately to mind: the relationship of the sympathetic system to voluntary muscle remains a problem of importance and interest for future investigations.

The pendulum, then, of anatomical and physiological thought regarding the sympathetic innervation of skeletal muscle has apparently swung too far, but it returns to the older conceptions which today appear to be real foundation-stones of knowledge. Obviously, however, these foundation-stones are not all laid, particularly in regard to the sensory innervation of voluntary muscle. Sherrington's thoroughly adequate and really splendid description of the mammalian muscle-spindle thirty years ago remains today a fairly complete summary of our present knowledge of this structure, but the muscle-spindle does not constitute the sole mechanism of sensory innervation of striated muscle. Other structures, such as the neuro-tendinous endings, were discovered twenty and more years ago—these non-encapsulated arborizations serve apparently the same function as do the spindles. Thus, in the extrinsic muscles of the eye the neuro-tendinous endings seemingly act as the receptors of the very delicate

muscle-sense of these structures, where almost all investigators have failed to find typical spindles. Yet in some of the muscle-groups of the mammalian body there is available no information regarding the kind of receptors for the sensory nerves; it is remarkable that almost nothing is known of the sensory mechanism of the "visceral musculature" of branchial arch origin. It would be extremely satisfactory, anatomically, if one could demonstrate that somatic musculature had one type of receptor and branchial another; this suggestion is rendered valueless, however, by the demonstration that the masseter muscle possesses true encapsulated spindles, as reported by Cipollone (1897) and Cuajunco (1926). Perhaps some other generalization, grouping muscles into different classes on the basis of their sensory innervation, may be possible in the future; today, such a generalization cannot be made.

It now appears, however, that we are on the verge of further important anatomical discoveries regarding the sensory mechanism of skeletal muscle. Last spring, Marion Hines described the occurrence, in certain somatic muscles of the frog, of slender striated muscle-fibers, gathered together in the form of a cylinder, with grouping of their nuclei in the center of the cylinder. The innervation of these slender fibers, according to Hines, resembles that of a true spindle, though the whole structure lacks a capsule. Experimental extirpations, carried out by this investigator, establish a sensory function of these structures. Work now in progress by Olive Cushing Smith suggests that somewhat similar structures may occur in mammalian forms, particularly in the facial musculature of cats; in this group of muscles the sensory mechanism has hithertofore been entirely unknown.

The possible difference in the anatomical types of sensory mechanism, indicated by these findings, calls attention at once to another of the significant problems of muscle and nerve—the sensory pathways of the so-called purely motor cranial nerves. Sherrington's analysis of the components of the nerves innervating voluntary muscles, showing approximately one-half of the fibers to be sensory, was soon followed by studies of the cranial nerves. The oculo-motor, the trochlear and the abducens nerves were within the next two decades found to have sensory fibers included in their midst, though the course of these fibers, their sensory ganglia, and their central sensory endings

are today almost unknown. Another of the so-called purely motor nerves in the cranial group—the hypoglossal—has recently been shown by Langworthy (1924) to be mixed in type, i.e., having both motor and sensory fibers. Langworthy's work on the tongue is a splendid example of modern anatomico-physiological investigation. Excluding the lingual and glosso-pharnygeal nerves by section, Langworthy was able to demonstrate an intact sensory mechanism; with the hypoglossal cut, the nerves to the muscle-spindles of the tongue disappear entirely. This work on the hypoglossal nerve leaves but one more important cranial nerve for functional analysis—the facial. Some progress in the solution of the problem of the sensory supply of the facial muscles has been made recently by Davis (1923), who showed that deep pain in these muscles of expression courses to the nervous system by way of the facial nerve and not by way of the trigeminal; the neurological surgeon has likewise demonstrated that muscle sense in this mimetic musculature is not lost after extirpation of the Gasserian ganglion or avulsion of its sensory root. Until information is available regarding the sensory structures of the facial muscles, final analysis of this nerve cannot be made. A more essential problem in anatomical neurology cannot today be undertaken, for upon its results will depend a great deal of clinical neurological diagnosis as well as surgical therapy.

The analysis of the various cranial nerves, carried out during the past quarter century and still actively being studied, may serve as a very good example of a field in which thirty years ago relatively complete and satisfactory knowledge held. The peripheral course of these cranial nerves had been well worked out; the investigation of their central connections, both by the degeneration method and by the comparative anatomical method, alone seemed to be necessary to complete our knowledge. Then with realization that each of the muscles of the head required a delicate sensory mechanism, anatomists quickly assumed that the adjacent or anastomosing sensory nerves took over this function. Thus, the ophthalmic branch of the trigeminal was considered as affording the pathway for muscle-sense from the extrinsic ocular muscles; the maxillary division, largely that for the muscles of expression; the lingual and the glosso-pharyngeal for the intrinsic muscles of the tongue; and so may the tale go on. The

difficulty here was the character of the foundation-stone of neurological knowledge: the purely motor nerve in the cranial group does not exist.

There is one further field of the relationship of muscle and nerve which requires comment, because of the extreme interest of recent work. This phase of the problem is that related to the plurisegmental innervation of individual striated muscle-fibers. Anatomists have known for many years that in the embryology of these muscle-fibers, several myotomes may contribute—in other words, the individual adult striated muscle-fiber may arise by the fusion of myoblasts from adjacent metameres. Such a fiber has long been known to possess more than one motor end-plate; each of these end-plates was assumed to be innervated by nerves from the neuromere corresponding to the myotome of origin of that part of the fiber. As a consequence of this metamerism, the successive end-plates should be innervated by successive segments of the nervous system.

Extension and possible correction of our conceptions of this plurisegmental innervation of striated skeletal muscle are largely due to the researches of Agduhr (1915). This investigator first showed, by calculations of the work done by a muscle innervated by two nerves, that there existed considerable overlap of peripheral innervation. This demonstration was effected by maximal stimulation of the two nerves, at first independently of each other and then together: the work done by the muscle on maximal stimulation of each of the nerves was greater than half of the work done on simultaneous maximal stimulation of the two nerves. The segmental overlap of innervation, as determined by electrical stimulation of the spinal roots entering into the formation of the peripheral nerves, was found by Agduhr to be greater than the peripheral overlap.

These findings suggested to Agduhr that at least some of the individual muscle-fibers composing the doubly innervated muscle must be innervated by fibers from different segments of the spinal cord. Cavalie (1901) and Floresco (1904) had previously identified motor nerves which gave terminal filaments to two or more end-plates on the same muscle-fiber or to adjacent fibers. In a second series of experiments, Agduhr (1916) performed intradural section of the seventh cervical and first thoracic nerves at 58 and 90 hours before killing the animal. This

experimental procedure left the eighth cervical nerve intact: subsequent histological study, by a modified silver impregnation method, revealed frequent individual striated fibers to be innervated by nerve-fibers from two adjacent segments of the spinal cord and occasional fibers to be trisegmentally innervated. In rare preparations, Agduhr showed an innervation of a single muscle-fiber by fibers from the seventh cervical and first thoracic nerves, with no evidence of innervation from the eighth cervical. Later, in a further series, Agduhr (1919) repeated his experiments, allowing longer intervals to elapse between his experimental sections: the results obtained were in every way in accord with his initial findings.

This idea of a plurisegmental innervation of individual muscle-fibers as brought out by Agduhr's work is not, as has been pointed out, a new conception, as it accords well with established embryological findings. Nor is it at all difficult to harmonize the idea of plurisegmental innervation with the presence of non-medullated nerve-terminations on the same type of muscle-fiber. Recent physiological observations on this subject really add but little to our fundamental conceptions; the problem is essentially anatomical and the demonstration of plurisegmental innervation is basically morphological.

The process of plurisegmental innervation has also been shown by Agduhr to hold for muscle-spindles as well as for the motor mechanism. Likewise, certain unpublished morphological findings by Fidel Cuajunco, regarding the embryological history of the innervation of muscle-spindles, support Agduhr's finding of plurisegmental innervation for these sensory structures in striated muscle: the necessary experimental work is now actively under way and in the course of the next few months, information regarding it will be available.

One phase of Agduhr's work on plurisegmental innervation is, however, disturbing to one of the generally accepted tenets in phylogenetic and ontogenetic development—the finding that an individual muscle-fiber may be innervated by nerves coming from segments not adjacent. Agduhr's description of the innervation of a single striated fiber by nerve-fibers from the seventh cervical and first thoracic segments, with no participation in the process by the eighth cervical, is striking, and no adverse criticism can be made of the experimental control or of the histological procedures. This finding is, however, utterly con-

fusing to our conceptions of embryological development, for it seems almost inconceivable that the myoblasts from two segments should fuse without participation of the intermediate segment in the process. It may be thought possible that the developing muscle-fiber innervated by one neuromere received a secondary connection at some time in its growth from a segment not adjacent, but such an idea of secondary innervation would be in direct opposition to one of the major theses of comparative anatomy—the hypothesis of Fürbringer.

In his epochal studies of the wing musculature of birds, Fürbringer (1888) presented data which indicated strongly that the relationship of a peripheral nerve to a striated muscle held throughout the differentiations or migrations of this muscle in phylogenetic development. This hypothesis that the peripheral nerve innervating a muscle furnishes a reliable guide to muscle homologies is of course opposed to any idea of a possible development of a secondary connection between nerve and muscle: it predicates a fundamental early and persisting relation between the primary neuroblasts and the final adult innervation. Though the concept of Fürbringer has been repeatedly attacked, it has withstood all criticism and today is fairly generally accepted. In comparative anatomical studies of musculature, the thesis has been of the greatest service, particularly in investigations of the important and difficult field of facial musculature. Here, thanks to the careful and important researches of Ernst Huber (1924), we have been furnished with what promises to be a classical example in support of Fürbringer's hypothesis. Huber's studies on the mammalian mandibulo-auricularis muscle demonstrate a migration from the neck portion of the primitive platysma, around behind the ear and thence downwards among the anterior facial muscles: through all of this migration the mandibulo-auricularis muscle pulls its nerve-supply from the posterior branch of the facial nerve. On reaching its final position, the mandibulo-auricularis muscle is pierced by filaments of the trigeminal nerve—an anatomical arrangement which led several workers to assume an innervation by this fifth nerve. Supporting his morphological studies by experimental procedures, Huber has been able to demonstrate that the mandibulo-auricularis muscle responds only to stimulation of the facial nerve; the trigeminal branches merely pierce the muscle without innervating it.

Such findings as these of Huber in support of the Fürbringer hypothesis make it extremely difficult to harmonize Agduhr's observation of the striated fiber innervated by the seventh cervical and first thoracic nerves. Other explanations than those already suggested to explain Agduhr's observation come to one: it is possible that the muscle-fibers in question were originally innervated by the three adjacent spinal nerves and that the second of the innervating nerves underwent total degeneration. And it is also remotely possible that the particular nerve-fibers innervating this individual striated fiber were really nerve-fibers from neuroblasts in near relation within the primitive spinal cord; these neuroblasts, however, sent fibers peripherally in more remote nerves. Much more research is necessary to settle these questions: there seems but little doubt that the hypothesis of Fürbringer will not be affected by the occurrence of plurisegmental innervations from segments not adjacent.

These, then, are some of the problems of the relationship of muscle and nerve. Scientific knowledge in this field has advanced rapidly in the last three decades, as it has in other branches of learning; but we are still today in the midst of an infinite number of unanswered questions regarding the anatomy and physiology of nerve and muscle. No attempt has been made to detail the many problems in this small corner of medical science: a few of the lines of apparently promising scientific research have alone been indicated. The topic affords however good examples of the process of accumulation of scientific knowledge:—the gathering of data, deductive reasoning and hypothesis from the data, and the subjection of these ideas to experimental proof. The particular phase of the nerve-muscle relationship which has been emphasized is that of the common meeting ground of the anatomist and physiologist: future advance in this subject will be achieved largely through coöperation of workers in these two subjects, or through the efforts of anatomists employing the weapons of the physiologists, and of physiologists using the methods of attack of the anatomists. Here is a great field of important research: today we stand on a ledge some way up the mountain-side of scientific knowledge of this subject. We feel that this ledge is safe—an integral part of the basic structure of the mountain—and from the apparent safety

of our footing we hazard a birds-eye view—a brief sketch of the present status of the problem—as we look downwards upon those slopes which patient research has overcome. But future work will find it necessary to test the security of the ledge upon which we stand: it may prove unsound, developing a fissure which will force a change in position; it may prove sound, so that from it we may ascend to higher levels of learning. Thus future investigation will search out the trails up the mountainside and it will, in its turn, make advances upwards, discovering new pathways, some good and some bad; yet in the end progress will be achieved and some day the perplexing problems of nerve and muscle, which now worry us, will be wholly solved, and science will be concerned with others as fascinating.

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THE COURSE OF URANIUM NITRATE INTOXICATIONS IN PREGNANT DOGS[1]

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Since the early studies of Leconte (1), which were later confirmed by Richter (2, 3), concerning the ability of salts of uranium to induce a renal injury, these substances have been employed by a number of students to produce an acute nephritis and to study the reparative processes which develop in the kidney that may finally lead to the production of a chronic nephritis. The uranium salts have been more frequently employed for such purposes than the salts of other heavy metals and bacterial products; probably because in certain of the lower animals, especially the rabbit, the acute renal injury may be accompanied by the development of an anasarca. Chittenden and Hutchinson (4) and Chittenden and Lambert (5) not only investigated the inhibitory effect of uranium salts on certain ferments, but they observed, in the early stages of their toxic action as kidney poisons, that the animals became polyuric and developed a glycosuria. The uranium glycosuria was studied at a much later date by Wallace (6) who considered it to be due to the renal injury and not associated with any increase in blood sugar. Schirokauer (7) and Heineke and Myerstein (8) induced severe vascular and epithelial injuries with uranium and noted the occurrence of the edema. Christian (9), Christian, Smith and Walker (10), and Christian and O'Hare (11), working largely with rabbits, described the renal epithelial injury from uranium and noted the occurrence of hyaline droplets in the walls of the glomerular capillaries. Pearce (12) classes uranium along with the chromates and considered the early damage to the kidney to be largely confined to the convoluted tubules. From the anatomical studies (13) of various nephrotoxic agents which have been made in this laboratory, we feel certain that, when uranium is used in the dog, the

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earliest evidence of a renal injury and the one which progresses with the course of the intoxication is of an epithelial character and very largely localized in the convoluted tubules. At a much later date than these investigations, the observation (14) was first made in this laboratory that the use of uranium in dogs induced a reduction in the reserve alkali of the blood and that the degree of the toxic effect of uranium was associated with the extent to which there occurred a disturbance in the acid-base equilibrium of the blood. Furthermore, in the same study, the observation was made that, if a solution of sodium carbonate was given intravenously to animals, the toxic effect of this substance was decreased to a marked degree. This final observation was later confirmed by Goto (15), who, in addition, studied the plasma chlorides, blood urea and the elimination of phenolsulphonephthalein in acute uranium intoxications.

From this brief and incomplete review of a few of the investigations concerned with acute uranium intoxications, it would appear that we possess in the salts of this metal a substance which can induce not only a local renal injury but probably a diffuse tissue disturbance which resembles in some of its manifestations the reaction obtained in certain toxaemias of pregnancy. It was for this reason that a uranium salt was selected to use in dogs during the gestation period.

Previous studies (16) of uranium intoxications have shown that the age of the animal influences to a marked degree its toxicity. The nephrotoxic effect and other manifestations of its action are usually more marked in old than in young animals. In old animals, there is often no period of polyuria but the output of urine is rapidly reduced. The urine contains a large amount of albumin, 3 to 8.7 grams per liter, numerous finely granular and coarsely granular casts, glucose, acetone and diacetic acid. Such changes in the urine may be rapidly followed by an anuria. Coincidently with the development of the albuminuria, and in a certain number of animals prior to this change, there occurs a reduction in the reserve alkali of the blood, a decrease in the elimination of phenolsulphonephthalein and later a retention of blood urea and non-protein nitrogen. A retention of creatinin finally occurs. Both the changes in the urine and in the blood are not dissimilar to the changes observed in certain toxic states developing during gestation.

In several recent investigations (17, 18), studies have been made on

74 pregnant dogs that varied in age from 11 months to 10 years and 4 months. The animals were observed during the fourth or fifth week of the period of gestation and in the terminal week. These studies, which have extended over a period of ten years, show that pregnancy in normal animals, exclusive of any evidence of a renal injury, tends to make unstable the acid-base equilibrium of the blood. The factor of the age of the animal and the duration of the pregnancy to a certain extent determine the stability of this equilibrium. The younger groups of animals more nearly maintained during the period of gestation a normal acid-base equilibrium than did the older animals, and furthermore, a larger percentage of the old animals showed a disturbance in this equilibrium early in the gestation period than did the young group of animals. In a final series of experiments (19), pregnant dogs of different ages were anaesthetized with ether for a period of two hours. Their ability to maintain a normal acid-base equilibrium of the blood during such a period was observed, the frequency of the development of a renal injury was noted, and in such animals studies were made of the ability of a glucose solution to maintain the acid-base equilibrium of the blood and aid in preventing the development of a renal injury. The experiments showed the inability of pregnant animals without the glucose protection to maintain during a two-hour period of ether anaesthesia a normal acid-base balance of the blood. The reserve alkali of the blood was reduced. The degree of reduction was more marked in old pregnant animals than in young animals. Finally, the degree of effectiveness of a solution of glucose in maintaining the equilibrium of the blood depended upon the age of the pregnant animal and the duration of the period of gestation. The stabilization of this equilibrium was more nearly effected in young pregnant animals than in old animals and it was of more value in such animals during the early weeks of gestation. With these observations in mind concerning the unstable character of the acid-base equilibrium of the blood during gestation, and with the knowledge of the ability of uranium nitrate to influence this equilibrium and also induce a renal injury, the following study has been made of the course of uranium nitrate intoxications in pregnant dogs.

Eleven pregnant dogs have been used in this series of experiments. The animals have varied in age from eighteen months to between three

and four years. The intoxications by uranium were induced at different stages of the gestation period in order to ascertain the influence of the duration of this state on the severity of the response by the respective animals. In the table of experiments the course of the intoxication is tabulated for five of the animals, which permits the variation in the severity and the difference in the terminations of the intoxications to be easily ascertained.

NORMAL OBSERVATIONS ON PREGNANT DOGS

Prior to the use of uranium, the animals were placed in metabolism cages and studied for four days in order to exclude the existence of a naturally acquired nephritis. The dogs were fed on scraps of meat, bread and butter and milk. They were given daily 500 cc. of water by stomach tube. Catheterized specimens of urine were examined qualitatively for albumin, glucose and acetone. Centrifugalized specimens of urine were studied for casts. The elimination of phenolsulphonephthalein in a two-hour period was determined according to the technique devised by Rowntree and Geraghty (20). The reserve alkali determinations were made by the method of Marriott (21), blood urea determinations by the method of Marshall (22), as modified by Van Slyke and Cullen (23), and the non-protein nitrogen and creatinin determinations by the methods of Folin and Wu (24).

A study of the table of experiments shows that all of the animals had a urine which was free from albumin and casts. Three of the eleven animals showed a trace of glucose in the urine. The urine of the same animals contained acetone. The two-hour elimination of phenolsulphonephthalein has been unusually high, especially when the subcutaneous method of administration is taken into account. It has varied from the maximum output of 91 per cent by the animal of Experiment 51, to the minimum output of 72 per cent by the animal of Experiment 14. Seven of the eleven animals had a normal alkali reserve of the blood which varied from 8.15 to 8.0. In the five remaining normal pregnant animals, the readings were below the normal minimum reading of 8.0. The readings on four successive days in one of these animals was 7.9. In the remaining four animals it was 7.95. The blood urea, non-protein nitrogen and creatinin determinations have been normal in all of the animals.

THE COURSE OF URANIUM NITRATE INTOXICATIONS IN PREGNANT DOGS

After having obtained the normal observations, the animals were given one subcutaneous injection of 4 mgm. of uranium nitrate per kilogram. Following this, the observations were continued, as previously described, on the second day after the use of uranium and on the sixth day if the severity of the reaction on the part of the animal did not demand an earlier study. In the animals that withstood the intoxication, observations were made on the tenth day and later at irregular intervals.

The table of experiments shows that with one exception all of the animals on the day following the uranium injection became polyuric. In the animal of Experiment 5, this was not the case. This animal was in the eighth week of the gestation period. The output of urine was rapidly reduced. At this stage of the uranium intoxication, all of the animals developed an albuminuria with numerous coarsely granular casts. The amount of albumin varied from 2 grams per liter, in the animal of Experiment 51, to 4.6 grams in the animal of Experiment 5. Glucose and acetone appeared in the urine at this early stage of the intoxication. The amount of glucose varied from a trace to 1.3 per cent in the animal of Experiment 14. The elimination of phenolsulphonephthalein by the pregnant animals at this early stage of the uranium intoxications is very variable and there is apparently no correlation between the amount of albumin per liter of urine and the elimination of this dye. In the animal of Experiment 51, two days after the commencement of the intoxication, the output of phenolsulphonephthalein had dropped from the normal elimination of 91 to 5 per cent. The urine contained 2 grams of albumin per liter. In the animal of Experiment 14, the elimination of the dye on the second day was 61 per cent as opposed to the normal elimination of 72 per cent. The urine of this animal contained 4.2 grams of albumin per liter. Without having a method to make an accurate observation, our feeling is that there is more nearly a correlation between the number of broad granular casts in the urine and the two-hour output of phenolsulphonephthalein than there is between the percentage elimination of the dye. In those animals with a sudden and marked reduction in the elimination of phenolsulphonephthalein, the urine,

TABLE I

The course of uranium nitrate intoxication in pregnant dogs

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	AMOUNT OF URINE IN TWENTY-FOUR HOURS	ALBUMIN PER LITER	GLUCOSE	ACETONE	PHTHALEIN IN TWO HOURS	R. pH	BLOOD UREA PER 100 CC.	NON-PROTEIN NITROGEN PER 100 CC.	CREATININ PER 100 CC.	LABORATORY NOTES
		cc.	*grams*	*per cent*		*per cent*		*mgm.*	*mgm.*	*mgm.*	
18	Normal observations	471	0	0	0	84	8.15	17	38	1.6	Female hound. Weight 11.4 kgm. Age 3–4 years. Duration of gestation, 4–5 weeks. Uranium nitrate 4 mgm. per kilogram
	Two days after uranium	892	3.2	1.02	Present	18	7.9	28	59	1.6	Animal does not appear sick. Takes some solid food. No vomiting. Premature labor
	Six days after uranium	1,042	1.1	Trace	Present, heavy	Trace	7.8	76	159	3.8	Refuses solid food. Refuses to stand up. Vomiting. Milk or water not retained. Diarrhea
	Ten days after uranium	435	Trace	0	Trace	14	7.95	38	70	1.2	Animal improved. Vomiting and diarrhea stopped. Retains milk and solid food
	Nine weeks after uranium	536	Trace	0	0	42	8.1	20	40	1.5	Animal appears normal. Has lost 2.1 kgm. in weight
51	Normal observations	831	0	0	0	91	8.1	15	32	1.6	Female collie. Weight 14.6 kgm. Duration of gestation 3–4 weeks. Uranium nitrate 4 mgm. per kilogram

51	Two days after uranium	1,411	2.0	0.79	Present	5	7.9	35	60	3.4	Animal appears in good condition. In general retains food. Vomits occasionally
	Six days after uranium	1,000	0.8	0.4	Present	5	7.85	21	58	3.5	Vomits frequently. Solid food and milk not retained. No diarrhea
	Ten days after uranium	812	0.8	0	0	32	8.0	21	53	1.5	Condition of animal improved. Does not vomit
	Two months after uranium	590	Trace	0	0	58	7.95	15	31	1.2	Animal appears normal. Loss in weight 1.7 kgm.
63	Normal observations	511	0	0	0	87	8.1	15	36	1.5	Female mongrel. Weight 12.2 kgm. 18 months old. Duration of gestation 4–5 weeks. Uranium nitrate 4 mgm. per kilogram
	Two days after uranium	836	2.7	0.76	Present	31	7.9	35	60	3.4	Animal appears bright
	Six days after uranium	947	2.8	0.3	Present, heavy	11	7.85	35	72	3.5	Animal refuses to eat. Vomiting. Diarrhea
	Ten days after uranium	311	1.1	0	Present, heavy	Trace	7.7	10	84	4.1	Animal unable to retain food or water. Refuses to stand. Premature labor. Seven puppies
	Twelve days after uranium	33	Heavy	Insufficient urine	Not made	0	7.7	10	86	5.3	Air hunger breathing. Muscular twitchings. Died in convulsions
14	Normal observations	547	0	0	0	72	7.95	12	28	1.2	Female mongrel. Weight 15.3 kgm. 3–4 years old. Duration of gestation period 5 weeks. Uranium nitrate 4 mgm. per kilogram
	Two days after uranium	1,096	4.2	1.3	Present	61	7.85	22	78	2.2	Animal appears sick. Down in cage most of time. Vomits. Diarrhea

TABLE I—*Concluded*

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	AMOUNT OF URINE IN TWENTY-FOUR HOURS	ALBUMIN PER LITER	GLUCOSE	ACETONE	PHTHALEIN IN TWO HOURS	R. pH	BLOOD UREA PER 100 CC.	NON-PROTEIN NITROGEN PER 100 CC.	CREATININ PER 100 CC.	LABORATORY NOTES
		cc.	*grams*	*per cent*		*per cent*		*mgm.*	*mgm.*	*mgm.*	
14	Six days after uranium	84	4.8	Trace	Present, heavy	10	7.7	11	92	4.1	Animal semiconscious. Slow, deep breathing. Surface cold. Vomits
	Seven days after uranium	0 Anuric	Not made	Not made	Not made	Not made	7.6	11	96	6.3	Animal died without convulsions. Air hunger
5	Normal observations	714	0	Trace	Trace	74	8.15	14	32	1.1	Female bull. Weight 18.4 kgm. 3–4 years old. Duration of gestation period 7–8 weeks. Uranium nitrate 4 mgm. per kilogram
	Two days after uranium	305	4.6	Trace	Heavy	8	7.7	78	74	3.8	Animal very sick. Down in cage. Vomits. Diarrhea
	Three days after uranium	105	4.6	Trace	Heavy	Trace	7.65	12	96	5.8	Animal unconscious. Blood obtained from heart. Air hunger. Convulsions. Death

regardless of the amount of albumin, has been rich in this type of cast. At the time of the appearance of albumin, glucose and acetone in the urine, there has occurred a reduction, or a further reduction, in the reserve alkali of the blood in all of the animals. The most marked initial reduction in the reserve alkali of the blood did not occur in one of the five animals in which the acid-base equilibrium of the blood was disturbed before the use of uranium. The most marked disturbance in this equilibrium developed in the animal of Experiment 5, between three and four years of age and in the eighth week of the gestation period. The animal had maintained during pregnancy a reserve alkali of 8.1. The use of uranium caused a reduction to 7.7. From this and other observations, it would appear that even if an old animal can maintain during gestation a normal acid-base equilibrium of the blood, the equilibrium is very readily upset by substances such as uranium and the general anaesthetics. By the second day of the uranium intoxication, there has occurred a retention of blood urea and of non-protein nitrogen. The creatinin retention at this period of the experiments is variable. Usually, the retention of creatinin develops later.

After the first two days of the intoxication by uranium in pregnant animals, the course of the experiments shows a certain marked difference, clinically, in the changes in the blood and urine and in their termination. These differences can be ascertained best by a study of experiments representative of these different types of response. Six of the eleven animals have shown a response to uranium which is indicated by the course of Experiments 18 and 51. In the animals of this group, following the initial response which has been described for the second day of the intoxication, there occurs by the sixth day a marked reduction in the amount of albumin and glucose in the urine. Acetone has continued to be present in the urine and the elimination of phenolsulphonephthalein has either remained stationary or has undergone a further reduction. The reserve alkali of the blood also shows a reduction from what it was on the second day of the intoxication. In the animal of Experiment 18, the reserve alkali was reduced to 7.8 on the sixth day and in the animal of Experiment 51, to 7.85. We have not been able to ascertain whether or not there is any connection between the acetonuria and the degree of reduction in the

reserve alkali of the blood. Except in one animal, the reserve alkali of the blood did not return towards the normal until after the urine had become acetone-free. At this stage of these experiments, the sixth day, the animals have shown a definite retention of blood urea, non-protein nitrogen and creatinin. The ratio of retention between blood urea and non-protein nitrogen is the one usually observed. The animal of Experiment 18 had, on the sixth day, a blood urea of 76 mgm. per 100 cc. of blood and a non-protein nitrogen of 159 mgm. The animal of Experiment 51 had a blood urea of 21 mgm. and a non-protein of 58 mgm. At this stage of the experiments, the animals show clinical evidence of a severe intoxication. They usually refuse to remain standing in the cages. Solid food is not taken and milk and water are vomited. A diarrhea is usually present.

Following the sixth day of the uranium intoxication, the six animals represented by Experiments 18 and 51 have shown a definite improvement and have returned towards the normal. By the tenth day following the use of uranium the urine has shown only a trace of albumin and has been free from glucose. Casts were present. Acetone has either disappeared or is present as a trace. The output of phenolsulphonephthalein increases and an improvement has taken place in the degree of disturbance in the acid-base equilibrium of the blood. Associated with these changes, the retention of blood urea, non-protein nitrogen and creatinin have diminished to normal values. All of the animals in this group with one exception gave birth to puppies at full term. The final observations on the animals of Experiments 18 and 51 were made at nine weeks and two months after the commencement of the uranium intoxication. The urine of both animals contained albumin and narrow hyaline and finely granular casts. These casts were not numerous. The urine was free from both glucose and acetone. The phenolsulphonephthalein output of the animal of Experiment 18 was 42 per cent, as opposed to the normal elimination of 84 per cent. The reserve alkali in this animal was 8.1. The normal reading before the uranium was 8.15. The animal of Experiment 51 had a normal phenolsulphonephthalein elimination of 91 per cent and a reserve alkali of 8.1. At the height of the uranium intoxication, the output of the dye was reduced to 5 per cent and the reserve alkali of the blood to 7.85. At the final observation, two

months later, the elimination of phenolsulphonephthalein was 58 per cent and the reserve alkali was 7.95. Clinically, the six animals falling in this group appeared normal except for a reduction in their weight. The animal of Experiment 18 lost 2.1 kgm., while the animal of Experiment 51 lost 1.7 kgm. These two animals that have been selected as representative of the first group of six animals in their response to uranium were killed following the final study. The autopsy findings of importance are as follows: The liver has shown evidence of repair especially in the zone of the peripheral part of the lobules. In this location, the liver cells appear as flattened cords with densely staining nuclei. The interlobular vessels are large and filled with blood. There is no increase in connective tissue. The liver cells at this late stage of the injury have not shown any evidence of degeneration. The changes of repair are marked. The kidney has shown less evidence of injury than would have been expected from the findings in the urine and in the blood. The glomeruli have shown an increase in nuclei and a matting together with some obliteration of the capillary loops The capsules have shown definite connective-tissue thickening. Subcapsular exudates have not been observed. The convoluted tubules have shown the most marked evidence of injury. The epithelium in some of the tubules is swollen and vacuolated, with poorly staining nuclei, while in other tubules reparative processes are marked. The cells contain mitotic figures, or the epithelium has been replaced by a low, flattened type of cell which is much less specialized in appearance than normal convoluted tubule epithelium.

The second group of five animals that were intoxicated by uranium have shown a response very different from the group of six animals just described. Three animals of this group (Experiments 63, 14 and 5) have been included in the table as representing the reaction of the latter group to the intoxication. A study of these experiments shows that all of the members of the group, with the exception of the animal of Experiment 5, became polyuric after the use of uranium. The urine contained from 2.7 to 4.6 grams of albumin per liter. Coarsely granular casts were very numerous. In the animal of Experiment 5, there developed at once a reduction in the output of urine from 714 to 305 cc. In the other animals of the group, following the initial polyuria there was a rapid reduction in the volume of urine. The

animal of Experiment 14 had a normal output of urine of 547 cc. which was increased on the second day of the intoxication to 1096 cc. On the sixth day there was an output of 84 cc. of urine. The animal was anuric on the seventh day. Both glucose and acetone appeared in the urine as early as the second day. At this time, when the urine contained albumin and was reduced in amount, the elimination of phenolsulphonephthalein was variable. In the animal of Experiment 14, the elimination of the dye was only reduced from the normal of 72 to 61 per cent, while in the animal of Experiment 5 there was a reduction at the same period of the experiment of from 74 to 8 per cent. The output of phenolsulphonephthalein in pregnant animals intoxicated with uranium nitrate shows no parallel with the amount of albumin in the urine or the degree to which the reserve alkali of the blood has been reduced. The most marked disturbance in the acid-base equilibrium of the blood occurred in the animal of Experiment 5. The reserve alkali was reduced from the normal of 8.15 to 7.7. A reduction of only 11 per cent developed in the elimination of phenolsulphonephthalein. As early as the second day in this second group of pregnant animals, there occurs a retention of blood urea, non-protein nitrogen and creatinin. It is of interest to note in three of these animals that from the commencement of the retention there is a disturbed ratio in the retention of urea and non-protein nitrogen. In the animal of Experiment 63, the blood urea at first increased to 35 mgm. with an increase in non-protein nitrogen to 60 mgm. By the tenth day of the experiment, the blood urea had dropped to 10 mgm. 5 less than the normal. At this time, the non-protein nitrogen had risen to 84 mgm. The disproportion between the urea retention and the non-protein nitrogen retention is even more pronounced in the animal of Experiment 14. The normal blood urea for this animal was 12 mgm. and the non-protein nitrogen was 28 mgm. On the seventh day of the experiment, the blood urea was 11 mgm., the non-protein nitrogen 96 mgm., and creatinin 6.3 mgm. At the present time, we have no explanation for this disproportion in the retention of these products unless it is due to the very severe liver injury and the much less pronounced renal injury. In addition to this difference in the retention of urea and non-protein nitrogen, this second group of five animals differs from the first group in that there is no evidence of an

attempt at a recovery. Acetone does not disappear from the urine. On the basis of the qualitative test, it appears to increase. In only one animal (Experiment 63) was there any reduction in the amount of albumin in the urine. The elimination of phenolsulphonephthalein was rapidly reduced to a trace and the reserve alkali of the blood was rapidly depleted to the low reading of 7.65 in the animal of Experiment 5, and to 7.6 in the animal of Experiment 14. The retention of creatinin has progressively increased in the usual manner. The increase is uninfluenced by the disproportion in the retention of blood urea and non-protein nitrogen. The highest retention of creatinin, 6.3 mgm., occurred in the animal of Experiment 14.

The clinical course of the intoxications in this second group of animals is different from the animals of the first group. The animals in the second group are severely ill as early as the second day of the intoxication. All of these animals have developed an air-hunger type of breathing and have then become comatose. Three of the five dogs died in convulsions.

The animals were autopsied immediately following death. The animal of Experiment 63 lived to the twelfth day of the intoxication. This animal showed a slight edema of the subcutaneous connective tissue. The condition was not observed in any of the other animals. Fluid was not found in any of the serous cavities. The liver and the kidneys have been the organs in which definite evidence of degeneration has been observed. In this group of animals in which there was no clinical or other evidence of improvement, the liver has shown an acute degeneration which is much more marked than are the changes of degeneration in the kidney. In the liver, there is no evidence of repair. The liver cells commencing at the periphery of the lobule are edematous, vacuolated and necrotic. The cells nearer the central vein fail to show as marked a degree of degeneration. In this part of the lobule, the cells are not so edematous and the nuclei stain fairly intensely. The intralobular capillaries are distended with blood. A study of sections from the livers of other animals has shown a more uniform degeneration. This is especially true of the liver of the animal of Experiment 5, that died in convulsions on the third day of the intoxication. In such sections, the entire liver lobule appears far advanced in necrosis. Many of the nuclei have disappeared and the

cell cytoplasm is so altered that it fails to stain. The sections have a mosaic appearance. The kidneys from such animals show a pathological response, which in degree is far less marked than the changes in the liver. The glomeruli show but little evidence of injury. The capillary endothelium appears swollen and the vessels are engorged with blood. The acute injury is largely localized in the convoluted tubules. The epithelium is edematous or vacuolated or far advanced in necrosis, while the epithelium of the other tubules, especially the cells in the loops of Henle, appear uninjured. In only one of the animals, Experiment 63, that survived for twelve days was there any evidence of repair.

CONCLUSIONS

1. Dogs during the period of gestation show a marked susceptibility to intoxications by uranium nitrate.

2. In such animals after the production of a marked intoxication, recovery may take place. The recovery is associated anatomically with the development of processes of repair in the liver and the kidneys.

3. In other animals the uranium induces a liver injury which is more severe and out of proportion to the severity of the renal injury. Such animals fail to recover. They develop an air hunger type of breathing and become comatose. A certain number die in convulsions. In only one animal of this group has there occurred any evidence of repair in the liver or the kidney.

4. Uranium intoxications in pregnant dogs are characterized by a marked albuminuria with granular casts. Acetone and glucose appear in the urine. The elimination of phenolsulphonephthalein is reduced. The reduction in the output of the dye is not proportionate to the amount of albumin in the urine. In such animals there occurs an early reduction in the reserve alkali of the blood and a retention of blood urea, non-protein nitrogen and creatinin. In one group of animals intoxicated by uranium, the ratio of retention of blood urea and non-protein nitrogen is atypical.

5. Dogs intoxicated by uranium nitrate during the period of gestation develop changes in the blood and urine, and may show a clinical course resembling certain of the major toxaemias of pregnancy.

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CARCINOMA OF THE CERVIX UTERI[1]

Its Operative Prognosis: A Clinical and Pathological Study to Ascertain the Prognosis Following Operation for Extirpation of the Malignant Process

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In a previous communication (1) it was shown that cancer of the cervix uteri varies greatly in malignancy. To recapitulate briefly, our study of the pathological material from 387 cases of cancer of the cervix uteri revealed that, aside from the adenocarcinomata, the epidermoid cancers could be divided into three large groups, each being designated according to the type of cancer cell predominating. There were, necessarily, various combinations of cell types encountered, but it was found that the predominant variety of cancer cell indicated the relative malignancy of the individual process. As a working classification, then, we have (1) spinal-cell cancer; (2) transitional-cell cancer; and (3) fat spindle-cell cancer.

To illustrate this distinct variation in malignancy of cancer of the cervix uteri the relative frequency of so-called "five-year cures," as reported in our first paper, occurring in each of the above types of cancer, is given in Table I, wherein are also compared the results obtained at the Mayo Clinic as reported by Broders.[2]

The results as shown in this table are so closely comparable as to stimulate skeptical speculation. However, it is worthy of consideration that the work in Rochester and in Baltimore was carried on independently and without knowledge on the part of either investigator that such a study was in progress elsewhere.

With such recent distinct advance in our knowledge of the variations in malignancy of this type of cancer the queston naturally arises as to whether or not certain fairly definite criteria can be established

[1] Submitted for publication, October 25, 1926.

[2] Personal communication.

which will make it possible to forecast with a reasonable degree of certainty the prospects for cure following operation for cancer of the cervix. Obviously any method designed to establish prognostic criteria possesses inherent limitations that with our present knowledge are insurmountable, and we fully realize that any scheme for prognosis, when applied to an individual case, may prove untenable.

We are also fully cognizant of the pitfalls encountered in trying to arrive at conclusions based on too small a number of cases. In the case of our group of adenocarcinomata and possibly to a somewhat less extent with the spindle-cell cancers we should be blatantly exposing ourselves to such a criticism had we ventured to accept without due reserve what appear to be our statistical conclusions. From a careful study of the pathologic and clinical phases of this

TABLE I

Percentage incidence of "five-year cures" for the different types of epidermoid cancer of the cervix uteri

	SPINAL-CELL CANCER (GRADE 2 OF BRODERS)	TRANSITIONAL-CELL CANCER (GRADE 3 OF BRODERS)	SPINDLE-CELL CANCER (GRADE 4 OF BRODERS)
	per cent	*per cent*	*per cent*
The Johns Hopkins Hospital (Martzloff).....	47+	24.2	9.5
The Mayo Clinic (Broders: Personal Communication).....	53.33	21.5	9.52

disease we have gathered certain facts which we have put in tabular form, in order to make up an orderly and composite picture of what would otherwise remain a scattered group of observations. In these tables, then, we have presented our findings merely for what they may be worth, we ourselves having remained totally ignorant of the ultimate outcome until the study was completed. Hence, in many instances what may appear in our tables to be statistical deductions must be accepted with due caution where the number of cases is small. It is hoped that our presentation may stimulate further observation, so that what in many instances can at present serve only as a suggestion, or possibly a working basis, may either be confirmed or disproved by the further observation of a sufficiently large number of carefully studied cases.

In the absence of any literature dealing with this phase of carcinoma of the cervix uteri based upon the considerations presented in this study, it is hoped that the findings presented will prove of value for the following reasons: (1) To provide criteria whereby some form of prognosis may logically be arrived at after operation; (2) to stimulate careful and detailed study of all specimens removed at operation, a procedure now almost totally lacking except in relatively few clinics; (3) to emphasize the fact that different types of cancer of the cervix uteri vary in malignancy just as do cancers of varying types in other situations.

PLAN OF STUDY

The present study is based on the cases of 145 patients suffering from unmistakable carcinoma of the cervix uteri in the Gynecological Service of the Johns Hopkins Hospital, and who were operated upon during the twenty-seven-year period between 1893 and 1920. In every case a panhysterectomy with the removal of a portion of the vagina and a wide parametrial dissection was performed.

Of the 387 patients studied in our first paper this group of 145 represents only those who fulfill the following criteria: (1) They were all operated on; (2) they all survived the operation and left the hospital alive; (3) their present status is known except in the case of some who, after being traced for five years, have been lost track of and have therefore been classed in the group of "five-year cures."

The necessity for such a selection of cases we believe is apparent when one reflects that we are in this study trying to ascertain what importance, if any, various factors studied possess for determining the ultimate outcome in a given group of carcinomata following operation. For the present, then, we are not concerned with the operative mortality but with the problem of whether or not criteria can be established that will enable us within reasonable limitations to forecast what the outcome may be in a patient who has survived the hazard of an operation.

The panhysterectomies performed were in the majority of instances effected through the anterior abdominal wall—the abdominal route. However, quite a few of the operations were performed wholly by the vaginal route and in some cases both the abdominal and vaginal

approaches (combined operation) were employed on the same patient. The two latter types of operation were used in the early days of the hospital when panhysterectomy for cancer of the cervix uteri was in its developmental stage. They have since been abandoned in favor of the abdominal operation which has been employed altogether in recent years. Doctor Cullen, however, feels that a vaginal panhysterectomy is still to be considered a desirable operation in the occasional case where an operable cancer occurs in the presence of a marked prolapsus, the uterus being outside.

In passing it may be noted that the operative mortality has shown a steady decline since the ultra-radical operations, as typified by the Wertheim procedure, were abandoned and our present operation was adopted.

Complete clinical records and all material removed at operation were available for this study. The pathological material was studied by making blocks from the entire length and antero-posterior thickness of the uterus, cervix and vaginal cuff when the two latter had not been entirely destroyed by cancer. The lateral extension of the process was studied by making blocks of the entire pericervical area including a wide margin of cervical tissue proper. These blocks were cut at right angles to the longitudinal axis of the cervix. Numerous microsections were taken from each block but no attempt to study serial sections was made.

The ultimate result in nearly every one of the patients studied is known. In the case of the occasional patient who has been lost track of, the fact will be specifically mentioned in its proper place.

In order better to appreciate the probable influence of the various factors that may possibly play a part in the determination of a prognosis, each group of cancers has been studied separately. A consideration of our results will probably convey the impression that they are entirely too favorable and not in keeping with the findings reported in our first paper, but it must be remembered that this study embraces only 145 patients, all of whom survived their operations, whereas in our former study our operative results are based on 203 patients regardless of whether or not they succumbed to operation.

Five-year cures. Under this heading we have included those patients who are still living and well (cures) as well as those who are now dead

but who lived five years or more after operation before succumbing to recurrent cancer or some other disease or were lost to our follow-up system.

SPINAL-CELL CANCER

Under this caption are included all of the cases where the spinal-cell type of cell is predominant irrespective of its combination with cells of other morphology, for it is from the predominating variety of cell that we foretell the degree of malignancy of the neoplasm. The cells characterizing this variety of cancer are morphologically similar to cells seen in the superficial zone of the stratum mucosum of normal stratified cervical epithelium. The cancer cells are usually polyhedral in shape with well defined cell outlines. The nuclei, many of which possess nucleoli, take only a moderately intense hematoxylin stain and are separated from each other by an abundant quantity of surrounding cytoplasm which is only lightly stained by eosin (Figs. 1, 2, 3).

In this group are included 30 patients, and of 28 the present status is known. Of the two exceptions one patient was followed for six years after operation and the other for ten months, after which they could not be traced.

In Table II are tabulated the cases of spinal-cell cancer, the salient data only being recorded. The bracketed numerals in smaller type which refer to individual cases make the tables cumbersome in appearance but this arrangement renders possible the tracing of the pathological and other data across the table and in the case of a "cured" patient to note at a glance conditions existing in that particular case.

All patients in this group not otherwise recorded are dead, death, with two exceptions, being due to the sequelae of cancer and occurring not later than two years after operation. This is a rather discouraging statement but one which emphasizes all the more the necessity for specific information rather than general impressions as our source of knowledge. Of the two exceptions, one patient died of pulmonary tuberculosis one year and the other of diabetes mellitus two years after operation.

In studying Table II one is immediately impressed by two out-

standing figures: first, the regrettably small number of cases, and secondly, the occurrence of "cured" patients. If one were to plot a curve of the "cures" in relation to the duration of symptoms before operation, one might conclude that it is just as well to operate late in the disease in spinal-cell cancer as to intervene during its incipiency. Such a deduction would hardly be justifiable; but nevertheless one cannot but be impressed by the high incidence of patients who are living and well in the group whose symptoms were of a duration of one to eight months.

Metastases. None of the patients in this group with demonstrable metastases have remained well, all dying of recurrence, though one, as may be noted, with metastases to the iliac glands, lived eight years before succumbing to a recurrence.

Extension into the pericervical tissues. All patients with definite extension of the neoplasm into the pericervical tissue, and we include here extension to the bladder and rectum, have died of recurrent cancer, but where the extension was questionable, as occurred in two instances, the patients are still in good health. Four (13.3 per cent) of these patients showed histological evidence of cancer extending directly beyond the radial confines of the cervix.

Extension to the corpus uteri occurred in 8 instances (24.2 per cent) and in 3 of these (37.5 per cent of 8) the patients are well, so that in this group of cancers, invasion of the corpus uteri is not incompatible with operative cure. However, a more critical analysis of this particular phase is even more relevant. For while in 8 patients in this group the cancer had invaded the uterus, in 3 it had extended also to other situations which in the light of our experience renders them beyond the scope of operative cure. Therefore, to evaluate correctly the possible rôle which uncomplicated uterine involvement may play, it would appear fair to deduct those cases from the total number (8) leaving 5 of these patients with carcinomatous extension limited solely to the uterus (vaginal involvement not being considered here). Of these 5 patients, 3, as before noted, are living and well, giving an operative cure of 60 per cent in those in whom the extension to the uterus is uncomplicated.

It would appear, therefore, that in carcinomatous extension from cancer of the cervix, that which is confined to the uterus is of least

TABLE II

Spinal-cell cancer

Duration of symptoms	Number of patients	Extensions or metastases								Extent of cervical involvement						Type of operation			D.C. several days prior to major operation	Radium	Epithelial pearls	Living and well		Dead or lost, but living or traced 5 years or more after operation	
		Ovaries	Iliac glands	Fallopian tubes	Uterus	Bladder	Pericervical tissues	Vagina +	Vagina ++	Length: One-third	Length: Two-thirds	Length: Entire	Thickness: One-third	Thickness: Two-thirds	Thickness: Entire	Combined	Vaginal P.H.	Abdominal P.H.				Number	Per cent	Lost or death not due to cancer	Recurrence
months																									
1	0																								
2	4							1 3	2 3, 4		1 4	3 1, 2, 3	2 1, 3		2 2, 4			4	1 3	2 3, 4	1 4	3 2, 3, 4	75		
3	2		1 1		1 1		1 1	2 1, 3			1 2	1 1		1 2	1 1		1 2	1 1	2 1, 2	0	2 1, 2	0	0		
4	7				3 4, 6, 7		1?	2 5, 7	3 1, 3, 4	2 1–5	2 3, 7	3 2, 4, 6	1 5	4 1, 3, 4, 7	2 2, 6		1 1	6 2, 3, 4, 5, 6, 7	1 6	1 5	6 1, 2, 3, 5, 6, 7	4 3, 4, 5, 6	57		
5	3							3 1, 3, 3		1 2	2 1, 3		1 3		2 1, 2			3	2 1, 3	0	1 3	2 1, 3	66.6		
6	2							1 2		1 1		1 2		1 1	1 3	1 1		1 3	1 3	0		1 1	50		

7	5			1	3	1	3	3	1		1	4		1	4			5	1	1	2	3	60		
				4	1, 2, 4	4	1, 4, 5?	2, 3, 4	1		3	1, 2, 4, 5		3	1, 2, 4, 5				3	5	1, 2	2, 3, 5				
8	2				1			1	1			2			2		1	1	1	0		1	50	This patient died 14 years after operation	
					1			2	1			1, 2			1, 2		2	1	1			2			
9	0																								
10	2							1		1		1	1		1			2	1	0	1	0			
								1		2		1	2		1				2		2				
11	0																								
12	3		1				1	2			1	2	1		2		1	2	0	0	1	0		1	1
			1				2	1, 3			2	1, 3	3		1, 2		3	1, 2			3			3	1
18	0																							(lost 6 years post-operative)	(8 years postoperative)
24	0																								

Key: Large numeral 2 indicates the numbers of patients. Small numerals $_{3}$, $_{4}$ each refer to an individual patient.

serious import. On the other hand, escape of the uterus apparently improves the post-operative prognosis, for, of the 14 patients who are cured, in 11 (78.8 per cent) there was no extension to the uterus.

Vaginal involvement. Where the vaginal involvement is not so extensive as to be *beyond operative removal* it appears that even a marked degree of it does not materially damage the prognosis, for of the 7 patients with extensive vaginal involvement 4 (57 per cent) are living and well, Of the 16 patients with only moderate vaginal involvement, in 3, other extensions negatived operative cure. Of the remaining 13, 7 (54 per cent) are well.

TABLE III

Showing radial involvement of cervix at different periods of disease in patients now living and well

DURATION OF SYMPTOMS	Thickness of cervix involved		
	ONE-THIRD (NUMBER OF CASES)	TWO-THIRDS (NUMBER OF CASES)	ENTIRE (NUMBER OF CASES)
months			
2	1	0	2
4	1	2	1
5	0	0	2
6		1	
7		1	2
8			1
Total.....	2	4	8

Extent of cervical involvement. In this group of spinal-cell cancers no definite relation can be shown between the extent of cervical involvement and the incidence of ultimate cures. Such findings hardly appear logical especially where the entire thickness is involved, for it would seem reasonable to assume that the deeper the involvement the more likely is peri-cervical dissemination to occur.

In Table III is summarily tabulated the extent of cervical thickness involved in the patients with spinal-cell cancer who are now in good health. The results appear to emphasize the *apparent unimportance of the extent of cervical involvement provided no pericervical dissemination occurs.*

Type of operation performed. Radical panhysterectomy through

the abdominal cavity was the operation performed on 25 (83.3 per cent) of these patients. Vaginal panhysterectomy was performed on 4 (13.3 per cent) and a combined vaginal and abdominal operation on one (3.3 per cent).

Of the patients who were submitted to the abdominal operation 12 (48 per cent) are living and well. One (25 per cent) of the four upon whom a vaginal panhysterectomy was performed is living, while the only patient in this group who had a combined operation is now in good health.

Duration of symptoms. What part the duration of symptoms plays in our scheme of attempting to foretell the ultimate outcome in a given group of cases is probably difficult to estimate in the spinal-cell group. We are arbitrarily selecting the eighth month of symptoms as the point beyond which a cure cannot be reasonably expected. However, in only 5 patients in this group were the symptoms more than eight months in duration and it can readily be seen from a glance at Table II that 2 of these 5 had none of the extensions that would ordinarily constrain us to consider them inoperable. It is then obvious that were there more patients in this group of cancers our data might be different. Fully appreciating the shortcomings involved we have, on the basis of our data, taken a symptom-duration of eight months as the time limit beyond which an operative cure for spinal-cell cancer may not be anticipated.

Diagnostic curettage several days prior to the radical operation was performed on 10 (33 per cent) of these patients. Three of these may be classified as cured, so that of the 14 "cured" patients, on 3 (21.4 per cent) we did a preliminary curettage.

For years it has been a routine procedure in this clinic to obtain tissue for microscopic examination before performing the radical operation. Our custom now is to examine frozen sections before proceeding with the extirpation. It happens occasionally that in some early cases the freezing method is not adequate for the preparation of sections sufficiently thin to permit of a microscopic diagnosis. In such instances the patient is put back to bed and nothing further is done until rapidly prepared celloidin or paraffin sections are available for examination. The importance of obtaining tissue for examination prior to the radical operation cannot be sufficiently stressed,

for it has proved of value in two ways, preventing, on the one hand, a serious operation where no cancer exists, and, on the other, occasionally revealing cancer where none was suspected (Cullen).

The use of radium is merely tabulated here and in the subsequent tables for the sake of completeness, no cognizance being taken of dosage, etc. Our experience with this form of therapy, we hope, may be the subject for a later report.

Miscellaneous. It will be noted that under "living and well" has been included one patient who died 14 years after operation, but whose death could in no way be attributed to a recurrence of her cancer. For this reason we consider it justifiable to consider this patient as having been clinically cured of cancer.

Two other patients who were operated during the twelfth month of their symptoms should be noted. One was lost track of after having been entirely well for six years after operation, the other after eight years died from the sequelae of a recurrence.

Cured patients. Five-year cures, so-called, are therefore seen to have been effected in 16 patients (53.3 per cent of 30) in the spinal-cell group, and permanent cures in 46.6 per cent. However, in analyzing Table II more carefully it will be noted that of the 30 patients forming this group, in 4 there was a definite pericervical extension of the neoplasm. One not included in the four just mentioned had iliac gland metastases, and three others, not included in the above, were beyond the eighth month of their symptoms before coming to operation. These eight patients, according to our criteria, were beyond the scope of a permanent operative cure.

It would be reasonable, then, to say that of the 30 patients in this group of cancers 8 belonged to the inoperable class. Therefore, of the 22 remaining patients who were *operable* 14 (63.6 per cent) are either living and well or may reasonably be considered as operative cures.

TRANSITIONAL-CELL CANCER

This is by far the most common variety. Grossly, it does not differ nor can it be distinguished from the other cancers in this situation. Microscopically, however, this tumor is distinctive, its cells resembling somewhat a well defined zone of cells seen in normal cervical epithelium

which is limited above by the characteristic spinal-cell layer and below by the distinctive single-celled basal layer. Because of their resemblance to this intermediate group of cells we have designated this variety of cancer as the transitional-cell type. These cancer cells have a very faint or undefinable cell membrane. The nuclei are round, take a deep blue hematoxylin stain, and are closely placed, being separated by a deep eosin-staining cytoplasm much less in quantity and more deeply staining than that separating the nuclei of the spinal-cell group. Nucleoli are commonly observed (Fig. 4).

In Table IV are tabulated the salient features in the cases of patients with the transitional type of cancer.

All but 6 of the 90 patients in this group, who were not living five years or more after operation, died within two years after operation. The six exceptions are 4 patients who died 3 years and 2 who died 4 years after operation. Five died certainly of recurrent cancer, but the cause of death in the sixth could not be ascertained. In fact 37 (41.1 per cent) of these patients died within the first year and a half following their operation, all but one from the sequelae following recurrence. The one exception (no. 4), who had had symptoms of two months' duration, died of a cerebral hemorrhage.

Metastases to the iliac glands were demonstrable in 4 patients of this group, all but one dying in less than one year after operation. This patient (no. 3), operated upon in the ninth month of symptomatic disease, died two years after operation. It may also be noted that this woman was the only one of the four to have a diagnostic curettage several days prior to the radical extirpation.

Metastases to the fallopian tubes were demonstrated twice in this group, both of the patients dying of recurrent cancer, though one lived six years before succumbing, five of these years being spent in complete comfort until the recurrence began to manifest itself.

Involvement of the corpus uteri by the cervical cancer occurred in 17 instances (18.8 per cent) in the transitional type of cancer, but in contrast to our results in the spinal-cell group, none of these patients may be classified as cured. Of these 17 patients, three lived two years or more following operation, one 4 years, one 2 years and one 3 years, the duration of their symptoms at the time of operation being, respectively, 5, 9 and 24 months. Of these 17 patients having exten-

TABLE IV

Transitional-cell cancer

DURATION OF SYMPTOMS	NUMBER OF PATIENTS	EXTENSIONS OR METASTASES								EXTENT OF CERVICAL INVOLVEMENT						TYPE OF OPERATION			D.C. SEVERAL DAYS PRIOR TO MAJOR OPERATION	RADIUM	EPITHELIAL PEARLS	LIVING AND WELL		DEAD OR LOST, BUT LIVING OR TRACED 5 YEARS OR MORE AFTER OPERATION	
		Ovaries	Iliac glands	Fallopian tubes	Uterus	Bladder	Pericervical tissues	Vagina		Length			Thickness			Combined	Vaginal P.H.	Abdominal P.H.				Number	Per cent	Lost or death not due to cancer	Recurrence
								+	++	One-third	Two-thirds	Entire	One-third	Two-thirds	Entire										
months																									
1	10						1 6	1 4	1 6	8 1, 2, 3, 4, 5, 7, 8, 9	1 10	1 6	8 1, 2, 3, 4, 5, 7, 8, 9	2 6, 10	0	1 1	4 2, 3, 9, 10	5 4, 5, 6, 7, 8	4 1, 2, 5, 10	0	3 5, 9, 10	5 2, 3, 4, 5, 9	50	2 7, 10 (8 years and 7 years respectively postoperative)	1 6 (6 years after operation)
2	6				2 3, 4		2 1, 4	1 1	1 4	2 2, 5	2 1, 6	2 3, 4	3 2, 5, 6	2 1, 3	1 4	1 1	0	5 2, 3, 4, 5, 6	3 3, 3, 5	0	0	2 2, 5	33.3		
3	16		1 4	1 9	2 1, 4		7 1, 4, 5, 8, 9, 13, 16	10 1, 2, 3, 4, 5, 6, 7, 8 10, 11	4 9, 12, 15, 16	1 8	7 2, 7, 9, 12, 13, 14, 16	8 1, 4, 5, 6, 8, 10, 11, 15	3 3, 9, 14	6 3, 6, 7, 11, 12, 15	7 1, 4, 5, 8, 10, 13, 16	2 3, 5	2 1, 2	12 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16	3 5, 12, 13	2 15, 16	3 4, 13, 14	0		1 2 (lost 10 years postoperative)	1 9 (died 6 years after operation)

4	14	1 8		1 12			4 1, 9, 13, 14	7 1, 3, 8, 10, 12, 14	3 6, 9, 11	2 4, 13	6 1, 2, 5, 7, 8, 14	6 3, 6, 9, 10, 11, 12	3 2, 4, 13	4 1, 3, 7, 11	7 5, 6, 7, 8, 9, 10, 12, 14	0	3 1, 2, 5	11 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14	5 2, 4, 7, 9, 12	3 9, 13, 14	0	4 3, 4, 11, 13	28.5		1 9 (died 5 years after operation)
5	7			1 6			2 1, 2	1 4	3 1, 2, 3		2 2, 4	5 1, 3, 5, 6, 7	1 4	2 5, 7	4 1, 2, 3, 6		1 1	6 2, 3, 4, 5, 6, 7,	3 1, 4, 6	1 7	0	2 3, 5	28.5		2 4, 2 (recurrence and death 5 and 7 years, respectively after operation)
6	11		1 5		1 6		4 4, 5, 6, 8	5 1, 2, 4, 9, 10	3 5, 6, 8	2 3, 11	3 1, 2, 8	6 4, 5, 6, 7, 9, 10	2 3, 11	4 1, 2, 7, 10	5 4, 5, 6, 8, 9	1 4	2 2, 3	8 1, 5, 6, 7, 8, 9 10, 11	4 4, 7, 9, 11	0	1 4	3 3, 9, 11	27.2	1 2 (died 10 years post-operative)	
7	4			2 3, 4			2 1, 2, 4	2 1, 2	2 3, 4			4 1, 2, 3, 4			4 1, 2, 3, 4			4 1, 2, 3, 4		0	1 4	1 2	25		
8	1											1			1		1		1	1		0				
9	8		1 3		2 1, 3	1 3	6 1, 3, 4, 5, 6, 8,	2 1, 3	3 3, 4, 5		2 2, 7	6 1, 3, 4, 5, 6, 8		2 2, 6	6 1, 3, 4, 5, 7, 8		2 1, 3	6 3, 4, 5, 6, 7, 8	5 1, 2, 3, 5, 6	0	1 3	0			1 6 (recurrence 7 years died 8 years after operation)
10	3		1 2		2 1, 2		2 1, 2	1 1	1 2	1 3		2 1, 2	1 3		2 1, 2	1 1		2 2, 3	0	0	0	1 3	33.3		
11	0																										

TABLE IV—*Continued*

DURATION OF SYMPTOMS	NUMBER OF PATIENTS	EXTENSIONS OR METASTASES							EXTENT OF CERVICAL INVOLVEMENT						TYPE OF OPERATION			D.C. SEVERAL DAYS PRIOR TO MAJOR OPERATION	RADIUM	EPITHELIAL PEARLS	LIVING AND WELL		DEAD OR LOST, BUT LIVING OR TRACED 5 YEARS OR MORE AFTER OPERATION	
		Ovaries	Iliac glands	Fallopian tubes	Uterus	Bladder	Pericervical tissues	Vagina	Length			Thickness			Combined	Vaginal P.H.	Abdominal P.H.				Number	Per cent	Lost or death not due to cancer	Recurrence
								+ ++	One-third	Two-thirds	Entire	One-third	Two-thirds	Entire										
months																								
12	5				2 2, 4		4 1, 2, 4, 5	3 2 1, 2, 3 4, 5			5		1	4 1, 2, 4, 5	1 1		4 2, 3, 4, 5	2 2, 4	0	0	0			1 3 (died 8 years post-operative of recurrence)
18	2				1 2		1 2	1 2			2 1, 2			2 1, 2			2 1, 2	0	0	1 2	0			1 2 (recurrence 10 years, died 12 years after operation)
24	3				2 1, 2		2 1, 2	2 1, 2			3 1, 2, 3			3 1, 2, 3		1 1	2 2, 3	0	1 2	2 2, 3	0			

sion of the cancer to the uterus, 3 (17.6 per cent) had no associated pericervical involvement. This absence, however, did not seem to indicate a more favorable outcome in so far as an ultimate cure was concerned.

Pericervical extension of the malignant process occurred in 37 instances (41 per cent) and was questionable in two. *All these patients, however, died of cancer.* In our previous communication we drew attention to the frequency of early pericervical involvement in this type of cancer and this point is again emphasized in this selected group of patients.

TABLE V

Patients with transitional-cell cancer with pericervical involvement living three years or more after operation before eventually dying of cancer

DURATION OF SYMPTOMS	DURATION OF LIFE AFTER OPERATION	CERVICAL INVOLVEMENT		RADIUM	PRIMARY D. AND C.	NUMBER OF PATIENTS
		Thickness	Length			
months	*years*					
3	6	One-third	Two-thirds	0	0	1
4	5	Entire	Entire	Yes	Yes	1
5	7	Entire	Two-thirds	0	0	1
9	8*	Two-thirds	Entire	0	Yes	1
12	3	Entire	Entire	0	0	1
18	12	Entire	Entire	0	0	1
24	3†	Entire	Entire	0	0	1

*No vaginal involvement.
†Limited vaginal involvement.

These patients, with the exceptions noted below, all died within 2 years after operation. In Table V are tabulated the patients who lived more than 2 years after operation before eventually dying of cancer. These patients were all submitted to an abdominal panhysterectomy and the notes on the use of radium and whether a curettage was performed several days prior to operation may be of interest.

Reference to Table IV will also show that all the patients in Table V, with two exceptions, had extensive vaginal involvement.

Vaginal involvement was extensive in 24 patients of this group. Only 2 (8.3 per cent) of these, however, may be classed as cured. In fact, of the 18 patients who are "well," only 6 (36.6 per cent) showed

cancerous invasion of the vagina, the involvement in 4 (22.2 per cent) of them being not extensive, while in two (11.1 per cent) the invasion was more wide-spread though removable.

In this connection it may also be worthy of note that of the 37 patients with pericervical invasion of the neoplasm 36 showed extension to the vagina. Conversely stated, of the 59 patients with carcinomatous involvement of the vagina, 36 (61 per cent) revealed extension to the pericervical tissues, thereby, according to our findings, being deprived of all hope of a permanent operative cure.

The extent of cervical involvement in relation to the "cured" patients is interesting in this group of cancers. In Table VI are grouped the "cured" patients to show the degree of cervical implication.

TABLE VI

Showing the radial involvement of the cervix in the cured patients with transitional-cell cancer

DURATION OF SYMPTOMS	THICKNESS OF CERVIX INVOLVED		
	One-third (number of cases)	Two-thirds (number of cases)	Entire (number of cases)
months			
1	5	0	0
2	2	0	0
4	2	2	0
5	0	1	1
6	2	0	1
7	0	0	1
10	1	0	0
Total.....	12(66.6%)	3(16.6%)	3(16.6%)

From this table it is readily seen that of the "cured" patients in this group twice as many had one-third or less of the cervical thickness involved in cancer as compared with those having more extensive involvement. These findings are considerably at variance with those for the spinal-cell type of cancer and are sufficiently striking to suggest that radial cervical involvement may be an important factor in determining the operative curability of the transitional type of cancer.

This is all the more vividly brought out when one considers the number of cured patients occurring in each of the groups into which the thickness of cervical involvement is subdivided. Of 21 patients in

whom one-third or less of the cervical thickness was involved in cancer, 12 (57.1 per cent) were cured. Of 23 patients who had two-thirds of the radius of the cervix invaded by cancer, 3 (13 per cent) are well, while of the 46 patients in whom the entire thickness was involved, 3 (6.5 per cent) may be considered cured.

These figures possibly lose somewhat their striking importance when one considers that during the first eight months of the symptoms the incidence with which one-third, two-thirds and the entire cervical

TABLE VII

Showing the relation of the extent of radial involvement of the cervix to the incidence of operable and cured patients with transitional-cell cancer

DURATION OF SYMPTOMS	ONE-THIRD OF CERVICAL THICKNESS INVOLVED IN CANCER				TWO-THIRDS OF THICKNESS INVOLVED IN CANCER				ENTIRE THICKNESS INVOLVED IN CANCER			
	Total number of cases	Operable cases	Cured cases	Cures in operable cases	Total number of cases	Operable cases	Cured cases	Cures in operable cases	Total number of cases	Operable cases	Cured cases	Cures in operable cases
months				*per cent*				*per cent*				*per cent*
1	8	8	5	62.5	2	1	0	0	0			
2	3	3	2	66.6	2	1	0	0	1	0	0	
3	3	2	0	0	6	6	0	0	7	1	0	0
4	3	3	2	66.6	4	3	2	66.6	7	3	0	0
5	1	1	0	0	2	2	1	50	4	1	1	100
6	2	2	2	100	4	4	0	0	5	1	1	100
7	0				0				4	2	1	50
8	0				0				1	1	Lost	
Total...	20	19	11	57.8	20	17	3	17.6	29	9	3	33.3

thickness, respectively, were involved was 95, 86, and 65 per cent. However, despite this, the figures in the preceding paragraph remain of dominant significance.

Table VII brings out plainly the situation existing in the group of patients seen during the first eight months of their symptoms, in so far as the extent of radial cervical involvement is concerned. One is impressed at once by the high incidence of cures in the operable cases and the relatively low incidence of operable cases among those patients

in whom the entire cervix was involved. It is also impossible not to be impressed with the fewness of cures in the group where the radial cervical involvement is limited to two-thirds, despite the high incidence of apparent operability, in so far as demonstrable ineradicable extension is concerned.

Type of operation performed. In this group 7 patients were submitted to the combined vaginal and abdominal panhysterectomy. All but three died in less than one year after operation. Of the 3 exceptions one lived four years and the other two, 2 years, the duration of symptoms before operation being, respectively, one month, two months and six months.

On 16 patients (17.7 per cent) a vaginal panhysterectomy was performed, and 4 (25 per cent of 16) are living and well. Three of these patients were in the first month of their symptoms, while the remaining one was in the sixth month.

The most common operation was the abdominal panhysterectomy, which was done in 67 instances (74.4 per cent). Of these patients, 14 (20.8 per cent of 67) are living and well.

The occurrence of five-year "cures" in the patients operated by either the vaginal or abdominal route is, respectively, 43.7 per cent of 16 and 34.3 per cent of 67 patients.

Curettage of the cervical neoplasm several days prior to radical operation was done on 30 (33.3 per cent) patients in this group. Of these, 7 (23.3 per cent of 30) are living and well, while 11 (36.6 per cent of 30) can be considered as belonging to the group of five-year "cures."

In other words, of the 18 patients who are living and well 7 (38.8 per cent) had been curetted several days prior to the complete extirpation.

Duration of symptoms. A word should be said here concerning patient no. 3 in the group whose symptoms are of ten months' duration. In this, as well as in our previous study, this case has caused difficulty from the standpoint of proper classification as to the duration of symptoms. Her history could well have been construed in such a way as to make the duration of her symptoms much shorter. This would also seem reasonable in view of the evident incipiency of the neoplastic process as indicated by our histologic findings. Were it not for the

exception just mentioned it would appear that every patient whose symptoms had extended over a period of eight months or more before operation was probably beyond the scope of an operative cure.

Cures. Of the patients in this group 18 (20 per cent) are living and well though this percentage could be raised to twenty-four if one were to consider cured those who died of natural causes or those that could not be traced after a period of ten years. This has not been done, however, these patients being grouped with those in the last column to swell the number of five-year cures which totals 30 patients (33.3 per cent) and comprises those living and well, as well as those living five or more years before dying or not being traceable.

Percentage cure in operable patients. The question necessarily arises as to what the ultimate results would be in patients who in the light of our experience may be considered to come within the scope of operability. In this group of cancers it would appear from our findings that in addition to the criteria established for the spinal-cell cancers one should add extension of the cancer to the corpus uteri as one other factor indicating the *possible* improbability of an operative cure.

Therefore if we consider inoperable, in this group of cancers, the 37 patients with pericervical extension of the growth, three patients with corpus uteri extension and one with ovarian involvement not included in the previously mentioned 37, we have 41 patients (45.5 per cent) out of the 90 who are definitely beyond the scope of a permanent operative cure. If we may also consider any patient whose symptoms are over ten months in duration as being beyond operative reclamation we may add 10 more to the inoperable group making a total of 51 patients (56.6 per cent) out of 90 who presumably are beyond the reach of an operative cure.

We may therefore reasonably consider 39 patients in this group to be operable. This then gives us a permanent operative cure in 18 patients or 46 per cent of those whom we consider as coming within the scope of a possible surgical cure.

SPINDLE-CELL CANCER

In our first communication the cancer cell characterizing this group of cancers was referred to as the "fat spindle type of cancer cell." This latter designation is unwieldy and it would probably be less

TABLE VIII

Spindle-cell cancer

Duration of symptoms	Number of patients	Extensions or metastases								Extent of cervical involvement						Type of operation			D.C. several days prior to major operation	Radium	Epithelial pearls	Living and well		Dead or lost, but living or traced 5 years or more after operation	
								Vagina		Length			Thickness											Lost or death not due to cancer	Recurrence
		Ovaries	Iliac glands	Fallopian tubes	Uterus	Bladder	Pericervical tissues	+	++	One-third	Two-thirds	Entire	One-third	Two-thirds	Entire	Combined	Vaginal P.H.	Abdominal P.H.				Number	Per cent		
months																									
1																									
2																									
3	4		1		2		2	1	2	1		3	1	1	2	1		3	4	0	0	1	25		
			1		3, 4		1, 3	4	1, 3	2		1, 3, 4	2	4	1, 3	2		1, 2, 4	1, 2, 3, 4			4			
4	1										1		1				1				0	0			
5	1					1	1		1			1			1	1			1			0	0			
6	2						1		2			2			2			2	0	1	0	0			
							2		1, 2			1, 2			1, 2			1, 2		2					
7	1							1			1			1			1		0	0	0	0			
8	2							1	1		1	1	1		1		2		0	0	0	0			
								1	2		1	2	1		2		1, 2								
9	2			1	2	2	2	2				2			2		1	1	0	0	0	0			
				1	1, 2	1, 2	1, 2	1, 2				1, 2			1, 2		1	2							
10	0																								
11	0																								
12	1				1		1	1				1			1			1	0	1	0	0			
18	2		1		2		2		2			2			2			2	0	0	0	0			1*
			1		1, 2		1, 2		1, 2			1, 2			1, 2			1, 2							1
24	1			1	1		1		1			1			1			1	0	0	0	0			

*Died 6 years postoperative.

cumbersome to refer to this cell as the *spindle type of cancer cell* and to designate this group as the spindle-cell cancers.

This group, forming the least common variety of the epidermoid cancers of the cervix, is composed of cells which, as their name implies, are spindle-shaped. Their nuclei assume a deep hematoxylin stain, are closely placed and separated by only a small quantity of eosin-staining cytoplasm. Nucleoli are not infrequently observed, but they are not common (Figs. 5, 6).

The spindle-cell group for this particular study comprises 17 patients. In Table VIII are tabulated the outstanding features characterizing the disease in this group of cancers.

Of the 17 patients, 11 (64.7 per cent) died within a year and a half after operation; in fact the majority died within ten months. Of the 6 remaining patients 2 fall into the group of five-year cures, while, of the remaining 4, 2 died within 2 years and 2 within 3 years after operation. Three of these died from the sequelae of recurrent cancer, while 1, a hopeless case (the patient in the second year of symptoms before operation), died of acute cardiac failure.

Ovarian involvement was not noted in any of these patients.

Iliac gland metastases were noted in two instances, in both associated with extensive cervical and vaginal involvement as well as extension into the pericervical tissues. One of these patients with symptoms of three months' duration lived 1 year after operation and the other, whose symptoms were of 18 months' duration, lived 6 years before dying from a recurrence.

Fallopian tube involvement occurred in 2 patients; in each instance the uterus and pericervical tissues were also implicated. One of these patients lived one year after operation and was lost trace of, while the other, whose symptoms were of 24 months' duration, lived 2 years before succumbing to a recurrence.

Involvement of the corpus uteri occurred in 8 patients (47 per cent) of this group. One of these patients (12.5 per cent of 8) is living and well, while another lived over 5 years before dying of a recurrence. It is noteworthy that with one exception all of the patients in this group with extension of the cancer to the corpus uteri also had pericervical involvement. The exception is the one and only patient in the group who is living and well. The uterine extension in her case was not associated with pericervical extension.

Involvement of the pericervical tissues occurred in 10 patients (58.8 per cent). All except one died of recurrent cancer, and with two exceptions (this includes the patient just mentioned) none lived over one and one-half years after operation. The two exceptions can be easily traced on Table VIII. One of these patients lived 6 years before succumbing to recurrent cancer, while the other patient with symptoms of 24 months' duration died two years after operation as the result of a cardiopathy (clinical diagnosis) there being at that time no clinical evidence of a recurrence.

Vaginal involvement in the carcinomatous process occurred in 15 patients or approximately 88 per cent of this group. Singularly, too, the 2 patients without vaginal involvement had also the least extensive involvement of the cervix and only one of these two lived three years after operation before succumbing to a recurrence.

It is also worth noting that of the 10 patients with involvement of the pericervical tissues all had vaginal involvement, extensive in 7 and moderate in 3, and in each instance there was involvement of the entire cervical thickness by the cancer.

From this phase of the pathology little can be deduced that might serve to determine anything of prognostic significance.

The extent of cervical involvement in this group is not particularly relevant in so far as ultimate cure or post-operative longevity are concerned, because no definite relation can be shown between them. This seems unlike what would *a priori* appear to be the case and undoubtedly merely serves to indicate the malignancy of the spindle-cell cancer. The only "cured" patient (surviving operation 7 years) in this group had the entire length and two-thirds of the thickness of the cervix invaded by cancer together with vaginal involvement, while in the same period of the disease is another patient (no. 2, in the third month of symptoms) with minimal cancerous involvement who died of recurrence after two years.

The type of operation performed and the use of radium are readily seen at a glance and are not particularly relevant except possibly to note that of the 10 patients who had abdominal panhysterectomies performed 40 per cent lived 2 years or more after operation, while out of the 5 submitted to the vaginal operation only one (20 per cent) lived as long. On only 2 patients in this group was the combined

vaginal and abdominal operation performed and one of these (no. 2 in the third month of symptoms) lived 2 years before dying of a recurrence.

Curettage of the neoplastic process several days prior to the radical operation for extirpation was performed in 5 instances, and the only patient in this group now living is one on whom this procedure was carried out.

Duration of symptoms. What influence the duration of the symptoms before operation exerts on the ultimate outcome in spindle-cell cancer is difficult to determine. The number of patients in this group is so small that any deduction would appear too arbitrary. It may, however, be noted that after the eighth month of symptoms every patient had pericervical extension of the neoplasm. This alone, as previously demonstrated, in our experience precludes all hope of an operative cure, and from that standpoint renders the case inoperable. However, a patient may live for several years in complete comfort before succumbing to recurrent cancer.

One might therefore presume that any patient with spindle-cell cancer of the cervix with a history of over eight months' duration, should arbitrarily be considered inoperable in so far as the possibility of a permanent operative cure is concerned.

Operable cases. The criteria for operability in the spindle-cell group of cancer are probably similar to those recognized for the spinal-cell group, in view of the fact that involvement of the corpus uteri by the carcinomatous process does not altogether exclude the possibility of an operative cure, provided, of course, that uterine extension is not associated with pericervical invasion.

It will be noted that all patients in this group with iliac gland or fallopian tube involvement also had extension of the process into the pericervical tissues. This is also true of the patients with bladder involvement.

We may consider the 10 patients with pericervical extension of the cancer as being beyond the scope of an operative cure. These 10 patients include those mentioned in the previous paragraph, and also those whose symptoms are over eight months in duration. Therefore of the 17 patients who are now under consideration 7 (41 + per cent) may be considered operable.

TABLE IX

Adeno-carcinoma

DURATION OF SYMPTOMS	NUMBER OF PATIENTS	EXTENSIONS OR METASTASES								EXTENT OF CERVICAL INVOLVEMENT						TYPE OF OPERATION			D.C. SEVERAL DAYS PRIOR TO MAJOR OPERATION	RADIUM	EPITHELIAL PEARLS	LIVING AND WELL		DEAD OR LOST, BUT LIVING OR TRACED 5 YEARS OR MORE AFTER OPERATION	
		Ovaries	Iliac glands	Fallopian tubes	Uterus	Bladder	Pericervical tissues	Vagina		Length			Thickness			Combined	Vaginal P.H.	Abdominal P.H.				Number	Per cent	Lost or death not due to cancer	Recurrence
								+	++	One-third	Two-thirds	Entire	One-third	Two-thirds	Entire										
months																									
2	1											1			1			1	0	0	0	1	100		
4	1	1		1	1		1	1				1			1		1		0	0	0	0			
6	1						1	1			1				1			1	0	0	0	0			
9	2						1	1				2			2			2	1	1	0	1	50		
							1	2											1	1		2			
10	1				1		1	1				1			1	1			0	0	0	0			
12																									
18	1								1			1			1			1	0	0	0	0			
24	2					1	1		1		1	1			2			2	1	1	0	1	50		
						2	2		1		1	2							1	2		1			

Cures in this group are of less frequent occurrence than in any of the preceding, only 1 patient (5.9 per cent of 17) being alive and well today. Five-year cures amount to 11.7 per cent and in this connection it is interesting to note the extensive involvement with metastases in the patient who lived six years after operation before succumbing to recurrent cancer.

In any consideration of cured patients we are necessarily concerned with the possibility of cure in those who are operable. In view of the fact that only 7 patients in this group come within the scope of operability, the one cured patient gives us an operative cure of 14 + per cent in the operable cases.

ADENOCARCINOMA

Nine patients in this study had adenocarcinoma as the type of neoplasm involving the cervix. Table IX gives the salient features encountered in this variety of cancer.

Adenocarcinoma is the rarest type of cervical cancer but unlike the rarest form of the epidermoid variety does not possess a similar degree of malignancy. All but two of the patients in this group who died of cancer lived 2 years or more after operation, one living 2, one 3 and two 4 years before succumbing to a recurrence. In brief, of the 6 patients in this group who died of recurrence, 4 (66 per cent) lived two years or more after operation.

Ovarian involvement occurred in one patient (11 per cent) operated upon during the fourth month of symptoms and was associated with tubal, uterine and pericervical extensions. *Tubal involvement* occurred in this case.

Corpus uteri. The neoplasm invaded the body of the uterus in two instances (22 per cent). In both there was associated pericervical extension of the cancer with complete involvement of the cervix, and the ultimate result was fatal. In this group, therefore, involvement of the corpus uteri was uniformly fatal and the outcome to be anticipated in view of the associated pericervical involvement.

It is here impossible to predict the influence that extension to the uterus without pericervical involvement might have on the final outcome in a given instance.

Bladder involvement was noted in one patient of this group whose symptoms were of twenty-four months' duration. The involvement was by direct extension and death occurred three years after operation and the employment of radium.

Pericervical extension of the neoplasm occurred in five instances (55.5 per cent). All died, the entire thickness of the cervix being involved in cancer in all five, while the entire length was involved in four instances. Length of life after operation in cases with pericervical involvement and other details are tabulated in Table X.

Vaginal extension of the cancer occurred in 6 patients (66.6 per cent). As may be noted from a study of Table IX, it was associated in three instances with extension of the cancer beyond the radial

TABLE X

Longevity of the five patients in this group with pericervical extension of the adenocarcinoma together with other closely related considerations

DURATION OF SYMPTOMS	NUMBER OF PATIENTS	VAGINAL INVOLVEMENT	CERVICAL INVOLVEMENT		D. AND C. BEFORE OPERATION	RADIUM	DURATION OF LIFE AFTER OPERATION
			Entire length	Entire thickness			
months							
4	1	Yes	Yes	Yes	0	0	5 months
6	1	Yes	0	Yes	0	0	2 years
9	1	0	Yes	Yes	Yes	Yes	6 months
10	1	Yes	Yes	Yes	0	0	4 years
24	1	0	Yes	Yes	0	0	3 years

confines of the cervix. Of the three remaining patients with vaginal extension of the cancer, 2 (66.6 per cent of 3) are living and well, while of the total of 6 patients with vaginal involvement 33.3 per cent are living and well, so that vaginal encroachment by adenocarcinoma of the cervix does not preclude an operative cure.

Extent of cervical involvement. The entire cervix, with two exceptions, was involved in cancer; indeed, of the 3 living patients in this group 2 showed this complete involvement, while one, although the entire thickness was involved, showed only its distal two-thirds invaded by the neoplasm.

Type of operation performed. The abdominal panhysterectomy with isolation of the ureters and with a wide pericervical dissection

(1) was the operation performed in 7 of these patients (77.7 per cent). Of the remaining two a vaginal panhysterectomy was performed on one, and the combined vaginal and abdominal operation on the other. In both the cancer had extended beyond the confines of the cervix, so that any operative method, according to the experience of our clinic, would not have produced a permanent cure. Incidentally, it may be noted that the patient, upon whom the combined abdominal and vaginal panhysterectomy was performed, lived four years after operation, despite the pericervical invasion.

Diagnostic curettage several days prior to the radical operation was performed twice in this group. One of these patients died several months after the radical operation, and the other is living and well today. Here again one may conclude that curettage not followed by immediate operation is not incompatible with an ultimate operative cure.

Duration of symptoms. It is difficult, in fact obviously impossible, from a study of Table IX to formulate any rule as to a time limit based on symptoms beyond which a case of adenocarcinoma of the cervix is to be considered inoperable. In this respect this type differs entirely from the others heretofore described.

Cures. Of this group 3 patients are living and well today, giving an ultimate "cure" of 33.3 per cent. Reference to Table IX shows the duration of symptoms before operation and it is little short of marvelous to note that one patient had had outspoken symptoms for more than two years. The symptoms in this instance were a profuse, malodorous, vaginal discharge and a well defined metrorrhagia attributable to no other demonstrable endometrial or myometrial abnormality. This patient is living 14 years after operation, and three years ago underwent a radical breast amputation for cancer.

Other than these three there are no 'five-year cures' in this group. None of the other patients lived more than four years after operation, all dying of recurrences within that time. Of the 6 patients who died of recurrent cancer, 4 (66.6 per cent) lived two or more years after operation, the remaining two dying within six months after leaving the hospital.

Table XI shows graphically the duration of symptoms and the length of life of these six patients after operation.

Cures in operable cases. Although 33.3 per cent represents the operative cures in this group of cancers, it does not indicate the outcome to be anticipated in patients who come within our conception of operability. A glance at Table IX will immediately reveal that five (55.5 per cent) of these patients had extension of the cancer beyond the radial confines of the cervix, thereby losing all chance of operative cure. The five cases just mentioned also include all other extensions and metastases except those involving the vagina.

TABLE XI

Duration of life in patients dying of recurrent adenocarcinoma

DURATION OF SYMPTOMS	POST-OPERATIVE LENGTH OF LIFE			
	6 months	2 years	3 years	4 years
months				
4	1			
6		1		
9	1			
10				1
18				1
24			1	

The incidence of operable patients in this group therefore is 44.4 per cent. The incidence of operative cure in these operable cases is 75 per cent, thereby surpassing even the spinal-cell cancers in the favorable prognosis that may be offered in the operable patients who survive their operations. This is a wholly unforeseen result and altogether unanticipated when the present study, was undertaken.

SUMMARY

This study was planned primarily to ascertain, if possible, the factors that influence the chances of an operative cure in a patient suffering with cancer of the cervix uteri, *provided she survives the immediate effects of the operation.* Certain features are here briefly indicated upon which a prognosis may possibly be based.

Extensions or metastases, when demonstrable in either the regional lymph nodes, the adnexa, bladder, rectum or pericervical tissues, in our experience render impossible an ultimate operative cure.

Extension of the cancer to the corpus uteri without extension elsewhere evidently impairs the chances of an operative cure, but by no means obviates it in spinal-cell cancer. In the transitional-cell type, extension to the corpus uteri apparently outrules any probability of an operative cure. No generalization on this phase is permissible in the adenocarcinomata or in the spindle-cell cancers. It might be mentioned, however, that in the latter group the only cured patient had extension of the cancer to the body of the uterus.

Extension to the vagina of the neoplasm in otherwise *operable* patients warrants a more unfavorable prognosis than uterine extension in the spinal-cell cancers. Other factors being about equal, it might be said that the prognosis for an ultimate operative cure for vaginal extension as compared to uterine extension is as 1 is to 1.4. This complication is most serious in the transitional-cell cancer and is least serious of all in the adenocarcinomata. No generalization is warranted for the spindle-cell cancers.

Duration of symptoms before operation. It would appear justifiable on the basis of our experience to say that in the spinal- and transitional-cell cancers a duration of symptoms exceeding eight months is sufficient to put the patient beyond the scope of an operative cure. In the case of the spindle-cell cancers our study would indicate that this time is probably too liberal. For the adenocarcinomata no such stringent or arbitrary duration of symptoms can be predicated as for the cancers of the epidermoid variety.

The degree of cervical involvement, provided no pericervical implication has occurred, appears to be a relatively unimportant factor in the spinal-cell cancers as well as in the adenocarcinomata. In the case of the transitional-cell cancers, however, the degree of radial cervical involvement immediately assumes an important rôle, so that of the cured patients those having one-third or less of the thickness of the cervix involved in cancer, as compared with those having more extensive involvement, are as 2:1.

In fact, patients with transitional-cell cancer seen during the first eight months of their symptoms, and who have only one-third of the thickness of the cervix involved in cancer, present an operability incidence of 95 per cent, and the cures obtained in this operable group are 57.8 per cent. This is in distinct contrast to an operability

incidence of 15 per cent and a cure incidence of 17.6 per cent in the patients having two-thirds of the cervix involved or an operability incidence of 31 per cent with a cure incidence of 33.3 per cent where the entire cervical thickness is involved.

No generalization can be made for the spindle-cell cancers.

Diagnostic curettage performed several days prior to the radical operation was done in ten patients with spinal-cell cancer, and 21.5 per cent of these are well today. Of the transitional-cell cancer patients who are living and well, 38.8 per cent were curetted several days prior to operation. In the spindle-cell cancer group five patients were curetted. This includes the only patient in this group who is well today. Curettage was performed twice in the group of adenocarcinomata and one of these patients is well at the present time.

Undoubtedly curettage for diagnosis several days prior to operation for radical extirpation does not render the prognosis hopeless.

Prognosis in operable patients. In the group of epidermoid cancers the spinal-cell cancer offers the most favorable prospect for operative cure. Provided our criteria of operability are accepted, a cure may be anticipated in 63.6 per cent of the operable cases of spinal-cell cancer.

The transitional-cell cancer comes next, offering the possibility of a cure in 46 per cent of its sufferers, while the spindle-cell cancers are the least hopeful of all, in that only a 14 + per cent cure is revealed in our study.

The adenocarcinomata, in so far as this study is concerned, offer the most hopeful outlook, in that 75 per cent of the operable cases may be considered cured. However, we must again point out the small number of patients in this group and the danger of unqualifiedly accepting deductions formulated on such premises.

CONCLUSION

It seems possible, on the basis of this study, to render a post-operative prognosis in carcinoma of the cervix uteri, provided that the tissue removed at operation is studied with sufficient care, and that the operation is performed by a surgeon adequately trained in the surgery of the female pelvis. In these circumstances a prognosis may

be ventured with as a rational basis for its promulgation as is possible for cancer of the lip and of the breast. However, in the case of the adenocarcinomata our findings must be applied with caution, because of the small number of patients available for study.

To Doctor Cullen are due my appreciation and thanks for his ever helpful criticism and interest, and the privilege of making this study.

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FIG. 1. SPINAL-CELL CANCER WITH EPITHELIAL PEARLS

FIG. 2. A HIGH POWER VIEW OF AN AREA IN FIGURE 1, SHOWING TYPICAL SPINAL CELLS WITH WELL DEFINED CELL MEMBRANE, POLYHEDRAL OUTLINE, AND A LARGE QUANTITY OF PALE EOSIN-STAINING CYTOPLASM

Two epithelial pearls at *a*

FIG. 3. A HIGH POWER PICTURE SHOWING TYPICAL SPINAL CELLS WITH A MITOTIC FIGURE AT *a*

FIG. 4. SHOWING THE CELLS CHARACTERISTIC OF THE TRANSITIONAL TYPE OF CANCER

FIG. 5. SPINDLE-CELL CANCER, SHOWING THE CELLS WITH THEIR LONG AND BROAD NUCLEI, CLOSELY PLACED AND WITH LITTLE INTERVENING EOSIN-STAINING CYTOPLASM

FIG. 6. SPINDLE-CELL CANCER

This section is from the same specimen as the section shown in Figure 5. At *a* are mitotic figures which are not very distinct in this picture.

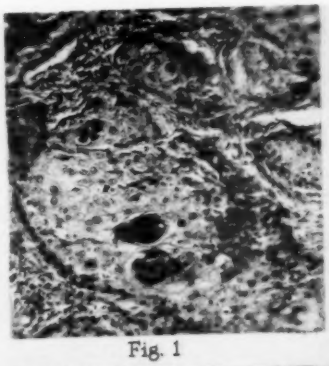

Fig. 1

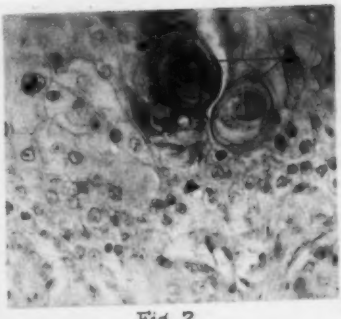

Fig. 2

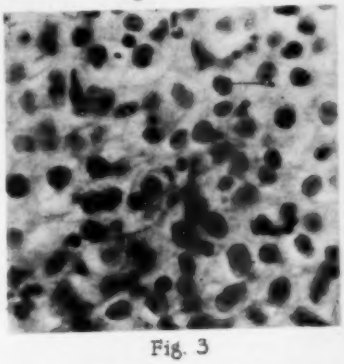

Fig. 3

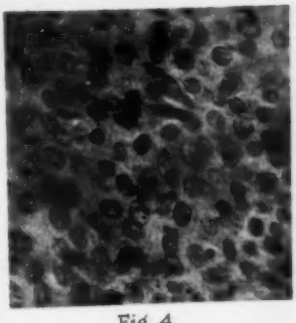

Fig. 4

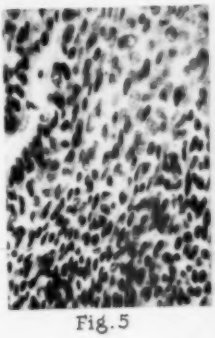

Fig. 5

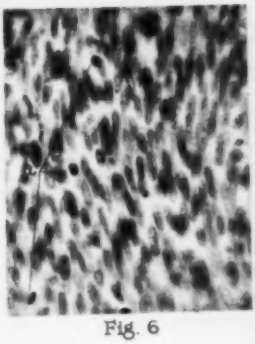

Fig. 6

BOOK REVIEWS

Modern Views on Digestion and Gastric Disease. By HUGH MACLEAN, M.D., D.Sc. 170 pp. $4.00. (*Paul B. Hoeber, NewYork, 1926.*)

This monograph presents in a clear, concise manner many of the more important views regarding digestion and gastric disease. As is customary, the author first devotes a chapter to the anatomy of the stomach, and in this section states that according to the experience of many observers the "acid control" theory regarding the regulation of the pyloric sphincter must be abandoned.

In the chapter on Physiology, he says that regurgitation of pancreatic secretion takes place during normal digestion; other than this, nothing particularly new is presented. He does not recommend the fractional method of gastric content extraction as a routine measure. In discussing the chief gastric diseases, he says that disturbances of innervation and of motor activity are important factors in the production of gastric symptoms, and that failure to digest food is seldom, if ever, a prominent feature; further, that it is by relieving spasm, and not by altering appreciably the acidity of the contents or the hypersecretion, that most of the symptoms associated with hyperacidity are overcome.

Under *symptoms*, he makes the well-timed statement that "hunger pain" is by no means pathognomonic of duodenal ulcer, and also emphasizes the point that in all cases of pernicious anemia true achylia is present.

In the discussion on gastric and duodenal ulcer, and gastritis, he differs sharply from those authorities who claim that many peptic ulcers are preceded or accompanied by pathological conditions in the appendix. He mentions the value of the detection of occult blood, but omits to state the importance of multiple investigations under varying conditions. He advances the opinion that the distinction between gastric and duodenal ulcers is purely arbitrary, and prefers the term "peptic ulcer" to designate ulceration in this area. The statement that duodenal ulcer never undergoes malignant degeneration, and that malignant degeneration of gastric ulcer is an uncommon occurrence seems rather dogmatic. He is convinced that a purely laboratory diagnosis (microscopic) of carcinoma, secondary to ulcer, is in most instances misleading. As might be expected, he offers no suggestion as to the etiology of carcinoma of the stomach, but is convinced that in the vast majority of cases the malignant process is primary, as evidenced by the length and character of the history. In his own office statistics, cancer showed an average of six and a half months' previous symptomatology; ulcer, an average of more than six years.

Much emphasis is laid on persistence of symptoms, occult blood, and the occurence of a large amount of lactic acid in the early stages of malignancy. The final chapters are devoted to a consideration of the diagnosis of gastric diseases, the examination of gastric contents, the radiological examination of the alimentary canal, and the treatment of the gastric diseases in general.

The book may be recommended to graduate and undergraduate students for ready reference regarding recent important findings. The undergraduate, however, would best use, in conjunction with it, the textbooks which deal more comprehensively with the various subjects.

E. H. G.

What's Best to Eat? By S. Henning Belfrage, M.D. (Lond.) M.R.C.S., L.R.C.P. With a practical supplement by Lucy H. Yates, M.C.A. 198 pp. $3.00. (*Wm. Wood & Co., New York, 1926.*)

The authors have aimed to afford a clear and accurate account of the most important principles which will aid in a more careful and intelligent selection of our daily food. In the first part Dr. Belfrage gives a scientific background sufficient to render possible a practical understanding of the various food factors in their relation to health. The second part by Miss Yates furnishes practical suggestions for the preparation of the most usual foods and of others less common by the use of which it is possible to avoid a monotony in diet. The conciseness and completeness of the work will render this little book valuable for those of the laity—and there should be many such —who are interested in proper nutrition and the conservation of health.

F. E. B.

Blood Chemistry: Colorimetric Methods for the General Practitioner, with Clinical Comments and Dietary Suggestions. Second Revised Edition. By Willard J. Stone. 129 pp. $3.25. (*Paul B. Hoeber, New York, 1926.*)

There is little that can be added to the criticism of the first edition of this manual made in these columns two years ago (Bull. Johns Hopkins Hosp., 1924, xxxv, 194). It is true that some of the newer methods have been added and the interpretative clinical notes expanded and made more comprehensive. But, on the other hand, the transcription of the details of the chemical methods still remains as inadequate and incomplete as it was in the first edition.

Manuals of this kind, when used by the general practitioner or by his technician, who is rarely a trained chemist, are actually a source of danger, as far as reliable results are concerned. It would seem much safer to present to the untrained analyst the complete detailed description of any given method instead of a much abbreviated "simplified" outline. The resulting simplicity is only of an apparent nature and would invariably lead to erroneous results.

It is still amusing to note that, while the user of this book has the choice of three methods for uric acid, he is left without a single quantitative method for estimating the degree of acidosis.

W. A. P.

Aviation Medicine. By Louis Hopewell Bauer, A.B., M.D. Published by authority of the Surgeon General. 241 pp. $7.50. (*The Williams & Wilkins Co., Baltimore, 1926.*)

This volume of 241 pages contains a good and useful introduction to a new field of practical medicine. It covers briefly but clearly the problems of selection of flying personnel. The physiologic, psychiatric, and other special technical studies necessary to determine fitness and disability, the care and maintenance of the flyer are well handled, and there is a valuable bibliography. Altogether, the book is recommended to physicians interested in the adaptation of man to flight and should be especially serviceable to those who would qualify for service as Flight Surgeons in the Reserve Corps or as examiners for civilian aviators.

T. R. B.

Light Treatment in Surgery. By DR. OSCAR BERNHARD of St. Moritz. Translated by R. King Brown, B. A., M.D., D.P.H., of London. 308 pp. $7.50. (*Longmans, Green & Co., New York, 1926.*)

This volume is probably the most complete work on this subject between the covers of a single book. Bernhard may be called the father of modern heliotherapy, as he has been utilizing the sun and its adjuncts, fresh air, rest, and diet for over thirty years. He has, in addition, made use of all of the various types of artificial sources of light.

The first chapter deals with the very interesting history of heliotherapy from the earliest ages to the present time. Then follows a through and detailed section on the physical, physiological and biological aspects of light. The effect of light on the body, and on the organs of special sense is discussed fully, both from the clinical and experimental points of view. A special section on the physiological effect of light on the eye has been contributed by Professor E. Wölfflin of Basel.

The important question of the pathological effects of sunlight both as regards conditions resulting from overdoses and from lack of sun or too little sun is thoroughly discussed and means of prevention are described.

The various forms of light therapy are then listed, among them thermotherapy, chromotherapy, actinotherapy and all the forms of artificial light sources. Results from all these types are compared.

The advantages and disadvantages of climate are described. Comparisons between lowland and mountain climates, between sea-shore and inland climates are made and the differences analyzed.

The second half of the book is devoted to treatment, indications and actual methods. All surgical conditions are considered, wounds; trophic disturbances of the skin and nerves, etc.; osteomyelitis; fractures, simple and compound; ulcers; blood diseases; and especially surgical tuberculosis. A large number of actual case reports are given. The results in infected non-tuberculous wounds of all kinds are almost startling. Many of us in this country are familiar with the results of heliotherapy in tuberculosis but few have seen such results as are described in the treatment of wounds. The treatment, of course, is combined with thorough cleansing of the wounds, by the use of antiseptics and mechanical means, and careful dressing. The section on tuberculosis is the last. This presents nothing

new, being much the same in general principle as that described in Rollier's book. In bone and joint tuberculosis conservative orthopaedic measures are employed in the prevention of deformities. Radical measures, such as joint excision, are used sparingly and only under the stress of special necessity, such as early return to work at the expense of a lost joint. Glandular, peritoneal and other forms of soft tissue tuberculosis are treated in the main conservatively.

The book presents a strong plea for the rational use of light not merely as an adjunct but as an important factor in the cure and prevention of disease, when combined with fresh air, diet and such special measures as we know to be of value in certain cases.

The bibliography given is immense and covers practically all of the work done on this subject in all countries in recent years. The book can be highly recommended to all and will prove of great value to special workers in the field of light therapy.

W. M. P.

BOOKS RECEIVED

Electrothermic Methods (Desiccation and Coagulation) in the Treatment of Neoplastic Diseases. Designed as a Practical Handbook of Surgical Electrotherapy for the Use of Practitioners and Students. By J. Douglas Morgan, B.A., M.D. Illustrated with 36 line and half-tone engravings. 1926. 12°. 172 pages. F. A. Davis Company, Philadelphia. $2.50.

Pathology and Treatment of the Inflammatory Diseases of the Nasal Accessory Sinuses. By Professor Dr. M. Hajek. Translated and edited by Joseph D. Heitger, A.B., M.D., and French K. Hansel, M.D., M.S. Fifth edition, completely revised and enlarged. 2 volumes. 1926. 8°. 702 pages. The C. V. Mosby Company, St. Louis. $17.00.

Outlines of Comparative Anatomy of Vertebrates. By J. S. Kingsley. Third edition, revised. With 435 illustrations largely from original sources. 1926. 8°. 470 pages. P. Blakiston's Son & Co., Philadelphia. $4.00.

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Surgery of Neoplastic Diseases by Electrothermic Methods. By George A. Wyeth, M.D. Preface by Howard A. Kelly, M.D. With one hundred and thirty-seven illustrations. 1926. 8°. 298 pages. Paul B. Hoeber, New York. $7.50.

Goiter and Other Diseases of the Thyroid Gland. By Arnold S. Jackson, M.D. With 151 illustrations. 1926. 4°. 401 pages. Paul B. Hoeber, New York. $10.00.

The Modern Treatment of Hemorrhoids. By JOSEPH FRANKLIN MONTAGUE, M.D., F.A.C.S. Foreword by Harlow Brooks, M.D., F.A.C.P. 116 illustrations. 1926. 8°. 296 pages. J. B. Lippincott Company, Philadelphia. $5.00.

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The Duodenal Tube and Its Possibilities. By MAX EINHORN, M.D. Second edition, revised and enlarged. Illustrated. 1926. 8°. 206 pages. F. A. Davis Company, Philadelphia. $3.00.

Practical Materia Medica and Prescription Writing. With illustrations. By OSCAR W. BETHEA, M.D., Ph.G., F.C.S. Fourth revised edition. 1926. 8°. 498 pages. F. A. Davis Company, Philadelphia. $4.50.

Cannula Implants and Review of Implantation Technics in Esthetic Surgery. In two parts. By CHARLES CONRAD MILLER, M.D. 1926. 12°. The Oak Press, Chicago. $2.00.

The Treatment of Fractures. With Notes Upon a Few Common Dislocations. By CHARLES LOCKE SCUDDER, A.B., Ph.B., M.D., F.A.C.S. Tenth edition, revised with 2027 illustrations. 1926. 8°. 1240 pages. W. B. Saunders Company, Philadelphia. $12.00.

Practical Dietetics for Adults and Children in Health and Disease. By SANFORD BLUM, A.B., M.S., M.D. Second revised edition. 1926. 8°. 362 pages. F. A. Davis Company, Philadelphia. $4.00.

The Surgical Treatment of Goiter. By WILLARD BARTLETT, A.B., A.M., M.D., D.Sc., F.A.C.S. With foreword by Dr. Charles H. Mayo. With 130 original illustrations. 1926. 8°. 365 pages. The C. V. Mosby Company, St. Louis. $8.50.

Defective Memory, Absentmindedness and Their Treatment. By ARNOLD LORAND, M.D. 1926. 8°. 340 pages. F. A. Davis Company, Philadelphia. $3.00.

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4

A NEW OPHTHALMOSCOPE[1]

Preliminary Report

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The image of an eye-ground as seen with an ophthalmoscope has a soft, blurred quality in which the finer details are obscured. Indeed the definition of the image falls far short of what would be regarded as satisfactory in most other optical instruments. The problem of improving the ophthalmoscope resolves itself into a consideration of the various factors which produce the blur in the ordinary image.

1. The spherical aberration of the normal eye is very considerable[2] and causes a blur in the ophthalmoscopic image which increases with the size of the observing aperture, and with any decentering of the observing system away from the axis of the observed eye. This aberration can be reduced to a minimum by using an observing aperture 2 mm. in diameter, carefully centered along the optical axis of the observed eye.

2. The chromatic aberration of the eye is about five times as great as this minimal spherical aberration and can be reduced to a negligible quantity, either by inserting a correcting lens into the observing system, or by limiting the spectral range of the light used to about one-fifth that of the visible spectrum. If such a spectral band is located near the violet end of the spectrum, the retina appears more or less opaque and a veil is cast over those structures that lie beneath its surface. This is true of the so-called "Red-Free Light." If the band is located at the red end of the spectrum, there is a loss of contrast between the retinal blood vessels and the general background of the fundus. The maximum definition can, therefore, be obtained by using yellow light of narrow spectral range.

[1] Received for publication February 14, 1927.

[2] See Ames and Procter, Jour. Amer. Opt. Soc., 1921.

3. A slight further gain in definition can be obtained by applying the method of oblique focal illumination which has been used to such advantage in the slit lamp corneal microscope. This aids greatly in the perception of variations of depth and elevation of details in the fundus.

An instrument has been constructed on these principles. The light from a ribbon filament bulb is first focussed upon a narrow slit. With the aid of a system of lenses and a mirror, an image of this slit can be focussed upon the observed retina. A color filter[3] is inserted into the illuminating beam yielding a light, the wave length of which lies between 550$\mu\mu$ and 625$\mu\mu$. By a suitable diaphragm, the illuminating beam can be greatly narrowed. This narrow beam can be decentered in relation to the observing system so as to produce oblique focal illumination of the retina, and also to extinguish the glare of light reflected from the surface of the cornea. By this means the eyeground can be examined through even an excessively narrow pupil, though the maximum definition of the ophthalmoscopic image cannot be obtained in such a case. By adjusting the decentration of the illuminating beam to the size of the pupil of the observed eye, the observing system is automatically centered along the optical axis of the eye.

The gain in definition of the ophthalmoscopic picture with this instrument is so great that a magnifying ocular can be used to advantage, and this has been supplied in the form of a small $2\frac{1}{2}\times$ magnifying telescope which is attachable on the observer's side of the ophthalmoscope yielding a total magnification of 37 diameters as against 15 with the ordinary ophthalmoscope. A fairly satisfactory view of some parts of the retinal capillary bed is thus obtained.

[3] Wratten Filters No. 52 (Naphthol Green) and No. 21 (Monobromfluorescine) combined.

DESCRIPTION OF A NEW ORGANISM THAT MAY BE A FACTOR IN THE CAUSATION OF PUERPERAL INFECTION[1]

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In the course of a study of the bacterial flora of the female generative tract during pregnancy, labor and the puerperium, we have encountered in six patients an organism which, so far as we know, has not heretofore been described. In five instances we were able to isolate this organism in pure culture and subject it to thorough bacteriologic study, while, in the sixth, the culture was lost before our work was completed but not before we had been able to identify it with the organisms obtained from the other five patients. Because of the similarities in the clinical histories of these patients as well as the marked resemblance of this organism to one which is apparently widely distributed among domestic animals, our findings should be of interest.

CLINICAL HISTORIES

Case I. Bact. No. XXXV, Obst. No. H 15,626. B. T. White, aged 43, primipara. Delivered by low cervical caesarean section 60 hours after the spontaneous rupture of the membranes and 24 hours after the onset of labor, at term, because of prolonged labor and lack of engagement of the head in an elderly primipara. At the time of operation the external os was dilated 2 cm. and the cervical canal was partly obliterated. No vaginal examinations were made during labor. The temperature and pulse at the time of operation were normal. The day afterwards the temperature rose to 100.6°F.; the following day to 101.8°F. and for the next twenty-two days varied from 100°F. to 101°F. On the eighth day after operation the abdominal incision broke down, through half its length, to the fascia and discharged large quantities of greenish, thin, foul pus. The wound gradually healed by granulation and the patient was discharged in good condition on the forty-first day after operation. From a culture taken at the time of operation from the lower uterine segment a pure culture of a long, thin, filamentous, gram-negative, anaërobic bacillus was grown.

[1] Received for publication February 21, 1927.

Case II. Bact. No. XLIV, Obst. No. H 15,898. L. C. Black, aged 25. Two previous pregnancies. Delivered by low cervical caesarean section because of pelvic dystocia after being in labor 41 hours with spontaneous rupture of the membranes at the onset. Two vaginal examinations were made during labor. At the time of operation the temperature was 101°F., pulse 120, and the external os was 4 cm. dilated with partial obliteration of the cervical canal. For the first four days after operation, the temperature varied from 102° to 103.4°, after which it gradually fell, becoming normal on the thirteenth day. On the fourth day after operation the lower third of the abdominal incision broke down to the muscle layer. There was a profuse discharge of thin, greenish, foul pus. The incision healed by granulation and the patient was discharged in good condition on the twentieth day after operation. From a culture taken at the time of operation from the lower uterine segment were grown a non-hemolytic anaërobic streptococcus, *B. welchii*, and a long, thin, filamentous, anaërobic, gram-negative bacillus. From the broken down abdominal incision a culture was made which showed the above described anaërobic streptococcus and the gram-negative, filamentous, anaërobic bacillus. Bacteriologically, these organisms were identical with those obtained from the culture taken at the time of operation.

Case III. Bact. No. XI, Obst. No. H 14,497. E. J. Black, aged 21, primipara. Delivered by low cervical caesarean section because of pelvic dystocia after a labor lasting 28 hours with spontaneous rupture of the membranes at the onset. Several vaginal examinations were made during labor. At the time of operation the temperature and pulse were normal and the cervix was fully dilated. The temperature varied between 101°F. and 104.6°F. for the first four days after delivery and then gradually fell, reaching normal on the eighth day. On the fifth day the lower third of the abdominal incision broke down to the peritoneum and large quantities of foul, thin, greenish pus were discharged. The wound gradually healed by granulation and the patient was discharged in good condition on the twentieth day after operation. From a culture taken from the lower uterine segment at the time of operation were grown *Staphylococcus albus*, an aërobic diphtheroid, a microaërophilic, non-hemolytic streptococcus and a long, thin, filamentous, gram-negative, anaërobic bacillus. Unfortunately, no cultures were obtained from the broken down abdominal incision.

Case IV. Bact. No. 8928, Obst. No. H 15,560. J. M. Black, aged 30, primipara. Wassermann positive, treated with arsphenamine during the last four months of pregnancy; otherwise the pregnancy was entirely normal. The labor lasted 18 hours and was terminated by low forceps. One vaginal examination was done at the end of the first stage of labor for the purpose of rupturing the membranes. The temperature and pulse during labor were normal. The temperature rose to 100.4°F. on the second day of the puerperium and varied between 101°F. and 103°F. until death on the twenty-first day. Physical ex-

aminations were negative until the thirteenth day, when a small, sensitive mass was palpable in the region of the left broad ligament. This mass became more definite on the fifteenth day, but did not point either in the vaginal fornix or in the region of Poupart's ligament. From the third day of the puerperium until death the vaginal discharge was profuse, greenish, thin and unusually foul. Aërobic blood cultures made on the twelfth and nineteenth days were sterile. An aërobic intrauterine culture on the fourth day was likewise sterile. The general condition of the patient gradually became worse. On the morning of the twenty-first day she became stuporous, with rapid respirations and pulse, and died at noon the same day. The anatomical diagnosis at autopsy done the day after death was: "Puerperal uterus. Abscess formation in left broad ligament. Slight generalized peritonitis. Extensive necrosis in liver (possible arsphenamine poisoning)." Cultures were made at autopsy from the heart's blood, the uterus and the broad ligament abscess. The blood cultures were negative. From the uterus were isolated *B. welchii*, *B. coli communis*, an anaërobic, non-hemolytic streptococcus and a long, thin, filamentous, gram-negative, anaërobic bacillus. From the pus in the broad ligament abscess the above-mentioned anaërobic streptococcus and anaërobic, gram-negative bacillus were identified. The bacteriological studies at autopsy were made by Dr. Irmgard Dresel, to whom we are indebted for a subculture of the gram-negative bacillus.

Case V. Bact. No. XVI, Obst. No. H 14,779. N. L. Black, aged 18, primipara, pregnancy normal. The patient was delivered by low forceps after a labor lasting 6 hours. One vaginal examination was made during the labor. The temperature and pulse were normal. On the second day of the puerperium the temperature rose to 101.6°F. and varied between 101°F. and 102.4°F. until the eleventh day, when it fell to normal and the patient was discharged in good condition on the sixteenth day after delivery. From the third to the twelfth days of the puerperium the vaginal discharge was profuse and unusually foul. From a cervical culture made one month before labor were isolated an aërobic diphtheroid, a non-hemolytic, anaërobic streptococcus and an anaërobic, gram-negative, long, thin, filamentous bacillus. Unfortunately, no cultures were taken during labor, and as there was an extensive laceration of the perineum, no intrauterine cultures could be obtained during the puerperium.

Case VI. Bact. No. S 17, Obst. No. 17,114. R. P. Black, aged 18, primipara. Spontaneous delivery at term. Duration of labor 16 hours 57 minutes (second stage 55 minutes). The membranes ruptured spontaneously during the second stage. No vaginal examinations were made during labor. The perineum was not torn. The puerperium was normal during the first two days. On the third day the temperature rose to 100.8°F.; fourth day 102.6°F.; fifth day 103.6°F.; sixth day 103.2°F.; seventh day 103.4°F.; normal thereafter. An intrauterine culture taken on the fifth day of the puerperium yielded an aërobic non-hemolytic diph-

theroid, an aërobic non-hemolytic streptococcus, and a slender, filamentous, gram-negative, anaërobic bacillus.

When the clinical histories of these six patients are compared, it is seen that they present several points of similarity. In the three who were delivered by caesarean section the abdominal incision broke down and discharged large quantities of thin, greenish, foul pus. The lochia in all six were identical in character. The convalescence of each patient was prolonged markedly beyond the usual time and all, except the one last mentioned, were seriously ill.

BACTERIOLOGICAL STUDY

When the organism described above as a long, thin, filamentous, anaërobic, gram-negative bacillus was first found, its resemblance to Actinomyces necrophorus (Löffler) was recognized, and for a time it was thought to be that organism. Throughout our bacteriological studies the strains isolated have been carefully compared with unpublished notes which one of us had previously made on studies of Act. necrophorus from animal sources and with parallel cultures of a typical strain of this organism obtained from Dr. W. A. Hagan of Cornell Veterinary College. Since this organism is practically unknown to students of human pathology and bacteriology, a brief account is here given.

Actinomyces necrophorus is well known to veterinary bacteriologists. It was discovered by Löffler (1884). A good description of the organism, the lesions in animals produced by it and references to the earlier literature are given by Jensen (1912). The organism is widely distributed in nature and produces characteristic coagulation necroses in many animals. These necroses, designated under the general term "necrobacillosis," may occur in almost any tissue of the body and are associated with such diseases as calf diphtheria, necrotic ulcers of the intestine in hog cholera, foot-rot of cattle and sheep, grease-heel or necrotic scratches and necrotic quittor of horses, lip-and-leg ulceration of sheep, multiple metastatic necroses of the liver and lungs of swine and cattle, and necrotic stomatitis of calves, lambs and pigs. The organism is sometimes present in avian diphtheria, in omphalophlebitis or the navel-ill of calves and lambs, and in oc-

casional spontaneous or natural infections of guinea-pigs and rabbits. Such an infection of the ear of a rabbit with a pure culture of the organism which was observed in our laboratory is shown in Fig. 1. Post-partum necrobacillosis of the uterus and vagina of cows is occasionally encountered and is almost invariably fatal. The infec-

FIG. 1

tion gains entrance through injury to the skin or any of the mucous membranes and may metastasize to any tissue of the body. A few cases of human infection are referred to by Jensen (1912) and by Shaw (1925). They include the following: two small abscesses on the fingers of persons handling laboratory animals infected with *Act. necrophorus* (Schmorl, 1891); in a nine months old child supposed to have died of laryngeal and naso-pharyngeal diphtheria, no diph-

theria bacilli were found, but an organism resembling *Act. necrophorus* was isolated (Ellermann, 1905); from an infection of the hand of a government meat inspector, Stemen and Shaw (1910) isolated *Act. necrophorus* in pure culture; an acute infection in man was reported by Van Wering (1923). So far as is known, no one has reported puerperal infection in women due to it.

From three human uteri at caesarean section, from one uterus and a broad ligament abscess at autopsy, from one intrauterine culture during the puerperium, and from one cervix during the last month of

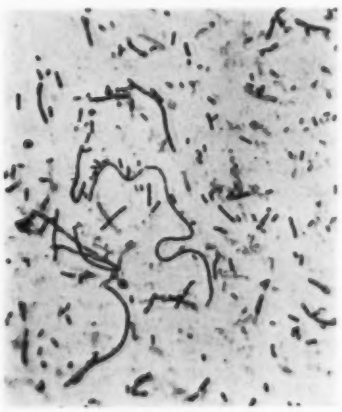

FIG. 2

FIG. 3

pregnancy we have isolated an organism which very closely resembles *Act. necrophorus* and for which the name *Actinomyces pseudo-necrophorus* is proposed. This is a non-sporulating, gram-negative, non-motile bacillus of irregular morphology. In stained preparations it often has a granular appearance. The diameter is not very uniform, and in length it varies from short almost coccoid forms to long filaments (cf. Fig. 2). Branching is occasionally seen. It is a strict anaërobe, and in fact is killed when surface colonies on blood agar plates or thin layers of fluid cultures are exposed to the air for from 40 minutes to 1 hour and 15 minutes. In plates of beef-infusion-agar containing about 8 per cent of human blood and incubated for at least 72 hours in an anaërobic jar (Brown, 1921), deep growth, when viewed under

a lens, appears as small, fuzzy colonies, usually without hemolysis or discoloration but occasionally with slight greenish discoloration of the surrounding blood cells. Surface colonies are small, round, dull, dirty white colonies with surface marking of concentric rings as the colonies become older. The edges are filamentous when viewed under the low power of the microscope. There is no hemolysis. A moderate number of gas bubbles may appear in the agar or between

TABLE I

Cultural characters and pathogenicity of strains studied

CULTURE	HEMOLYSIS	GLUCOSE			LACTOSE	SUCROSE	MALTOSE	MANNITOL	SALICIN	INULIN	PLAIN MILK	PEPTONE MILK	PATHOGENICITY		
		Amount of gas	Per cent CO_2	pH									Rabbit	Guinea-pig	Mouse
Act. necrophorus (Hagan)	+	0.4*	38	6.2	6.5	6.4	6.3	7.0	6.4	7.0	−†	+‡	+	−	−
Act. pseudo-necrophorus XI	−	0.15	27	6.3	6.9	6.5	6.4	7.0	6.5	7.0	−	−	(+)§		−
Act. pseudo-necrophorus XXXV	−	0.15	15	6.5	7.1	6.6	6.6	7.1	6.5	7.1	−	−	+	−	−
Act. pseudo-necrophorus 8928	−	0.2	11	6.4	6.9	6.5	6.5	7.0	6.6	7.0	−	−	+	−	+
Act. pseudo-necrophorus XLIV	−	0.5	20	6.4	7.0	6.6	6.5	7.1	6.5	7.1	−	−	−		−
Act. pseudo-necrophorus S 17	−	0.25	14	6.3	7.3	6.6	6.6	7.2	6.6	7.3	−	−	+		−

All strains produced a moderate amount of indol. None reduced nitrate to nitrite. None coagulated serum mixed with an equal amount of broth. None liquefied gelatin. None digested cooked meat.

* Total amount of gas measured in terms of the volume of medium, *e.g.*, 0.4, indicates that from each cc. of medium 0.4 cc. of gas was produced.

† Milk not coagulated.

‡ Peptone milk slightly coagulated.

§ Lesion produced but not fatal.

the agar and the bottom of the Petri dish. When grown in deep agar shake-tubes small fuzzy colonies appear below about 1 cm. from the surface, and there are many small bubbles of gas. The organism grows well in cooked meat, in serum broth, and fairly well in milk, especially if a little peptone is added to the latter; all of these media being covered by a layer of vaseline. Cultures have a foul odor similar to that of Act. necrophorus. The fermentation reactions are listed in Table I. These were carried out in cooked meat media or in serum broth to which was added aseptically 1 per cent of the

various carbohydrates. In these media more or less gas was produced. In glucose media from 0.15 to 0.5 volume of gas was produced as measured by the method of Brown (1922), and consisting of from 11 to 27 per cent carbon dioxide. The final hydrogen-ion concentration was always low (pH 6.3 to 6.6) and was the same at the end of two weeks as at the end of one week. Glucose, maltose, sucrose, and salicin were fermented; while lactose, mannitol and inulin were not. A moderate amount of indol was produced in a mixture of cooked meat and broth. Nitrate was not reduced. Human serum mixed with an equal volume of sugar-free broth was not coagulated. Gelatin was not liquefied, and the meat particles of cooked meat were not noticeably digested.

PATHOGENICITY FOR ANIMALS

In a few instances we have succeeded in infecting animals with this organism. A rabbit injected subcutaneously with 1.0 cc. of the fluid from a cooked meat culture of strain XXXV lost 60 grams in weight over night, and another rabbit similarly injected with strain S 17 lost 50 grams, but no local or general infection developed in either case. Rabbits injected intravenously with 1.0 cc. of culture invariably became very ill and lost from 50 to 200 grams in weight within 24 or 48 hours, but usually gradually recovered without evidence of infection. Two rabbits injected intravenously with strains XXXV and 8928 died within a few hours. From one of them the organism was recovered in pure culture from the heart's blood, but it may be that this should be regarded as a survival of the organism in the blood stream rather than as a true infection. On the other hand, a rabbit injected intravenously with strain S 17 died on the fourth day, after having lost 305 grams in weight. At autopsy no gross lesions were seen, but the organism was recovered in pure culture from the heart's blood and liver. A guinea-pig inoculated subcutaneously with strain XXXV showed nothing more than a transitory loss of 25 grams in weight. White mice were injected intraperitoneally with 0.5 cc. of each of the cultures. Two of them died, one in 24 hours and the other after 9 days. In the peritoneal exudate of both the organism was seen, but gram-positive cocci were also present, and it may be added that the immediate symptoms suggested toxemia

rather than infection. An attempt was made to immunize a rabbit by giving it several intravenous injections of living culture XXXV at intervals of 5 days. The loss in weight following injections became less with each succeeding injection. No infection developed. The serum of this rabbit before (normal serum) and after immunization (immune serum) was used in the experiment recorded in Table II. In this the filtrate injected intravenously into rabbits either alone or mixed with normal or immune serum was a Berkefeld filtrate from cooked meat cultures of strain XXXV. When it was injected alone or mixed with normal serum, the loss in weight was nearly twice as

TABLE II

Toxicity of filtrate of Act. pseudo-necrophorus XXXV

RABBIT NUMBER	INJECTED INTRAVENOUSLY WITH	WEIGHTS				TOTAL LOSS
		Before injection	After injection			
			24 hours	48 hours	5 to 7 days	
						grams
1	2 cc. filtrate	860	795	795	860	65
2*	2 cc. filtrate + 1 cc. immune serum	860	825	805	815	55
3	2.3 cc. filtrate + 2.3 cc. normal serum	1,600	1,485	1,515	1,590	115
4	2.3 cc. filtrate + 2.3 cc. immune serum	1,635	1,570	1,585	1,615	65
5	2 cc. filtrate	925	850	835	875	95
6	2 cc. filtrate + 1.5 cc. immune serum	955	900	925	975	55

* This rabbit was found to be suffering from an intercurrent infection.

great as when it was mixed with immune serum, thereby indicating that the immune serum possessed a certain neutralizing power for some toxic constituent of the filtrate.

For comparison with the strains of *Act. pseudo-necrophorus* there is also listed in Table I the Hagan strain of *Act. necrophorus*. A microphotograph of this strain is shown in Fig. 3. It is so nearly identical with the pseudo-necrophorus that only the differences need be mentioned. In carbohydrate media both organisms produce about the same final hydrogen-ion concentration. However, necrophorus ferments lactose, which is not attacked by pseudo-necrophorus. *Act. necrophorus* did not coagulate plain milk but in peptone milk pro-

duced a partial coagulation with a hydrogen-ion concentration of pH 6.2. In anaërobic blood agar plates necrophorus produced beta zones of hemolysis with rather poorly defined borders but with complete hemolysis next to the deep colonies. It was markedly pathogenic for rabbits, and in one animal the subcutaneous injection of a small bit of deep agar shake-culture caused death in nine days. Autopsy showed the characteristic extensive purulent coagulation necrosis of the subcutis and of the muscular layers of the abdominal

TABLE III

Attempt to immunize a rabbit against Act. necrophorus

DATE	WEIGHT	INJECTED INTRAVENOUSLY
	grams	
January 15	1,585	0.5 cc. culture exposed to air 1 hour. Killed
January 16	1,475	
January 21	1,625	1.0 cc. culture exposed to air 45 minutes. Killed
January 22	1,575	
January 26	1,650	1.0 cc. culture exposed to air 30 minutes. Killed
January 27	1,600	
January 30	1,710	1.0 cc. culture exposed to air 20 minutes. Killed
February 1	1,650	
February 4	1,660	1.0 cc. culture exposed to air 15 minutes. Not killed.
February 5	1,625	Rabbit appears well but has abscess in fore-leg. Aspirated pus yields Act. necrophorus in pure culture. Drew sample of blood for agglutination tests
February 11	1,500	Abscess discharging. Rabbit quite ill
February 14	1,325	Rabbit dead

wall about the site of injection, but the peritoneal surface was not involved and no foci of infection were found in other parts of the body. The heart's blood was sterile but Act. necrophorus was present in large numbers and in pure culture in the local lesion. The animal had lost 450 grams since inoculation. A rabbit injected intravenously with 1.0 cc. of fluid from a cooked meat culture lost 160 grams and developed a necrophorus abscess of the foot, but survived. Moreover, another rabbit injected intravenously with 0.5 cc. of culture

died in five days with a loss of 390 grams in weight. At autopsy many areas of necrosis were found in the muscles and various organs. A rabbit injected intravenously five times, at first with air-killed cultures and finally with a living culture, eventually died after having developed a large necrophorus abscess of the fore-leg (see Table III). A guinea-pig inoculated subcutaneously with 0.5 cc. of a cooked meat culture, and a mouse inoculated intraperitoneally with 0.5 cc., both survived without evidence of infection.

TABLE IV

Titration of agglutinins in serum of rabbit immunized against Act. pseudo-necrophorus XXXV

CULTURE	IMMUNE SERUM						
	1:10	1:30	1:90	1:270	1:810	1:2430	1:7290
Act. pseudo-necrophorus XXXV		+++	++++	++++	++++	+++	++
Act. pseudo-necrophorus XI	+++	++	+	sl.	sl.	sl.	
Act. pseudo-necrophorus 8928	++	++	++	++	+	+	+
Act. pseudo-necrophorus XLIV	+	+	sl.	sl.	sl.	sl.	
Act. necrophorus (Hagan)	++	+	+	+	+	+	+

CULTURE	CONTROL SERUM			SALT SOLUTION
	1:10	1:30	1:90	
Act. pseudo-necrophorus XXXV		++	+	sl.
Act. pseudo-necrophorus XI	++	+		sl.
Act. pseudo-necrophorus 8928	++	+	+	+
Act. pseudo-necrophorus XLIV	sl.	+	+	sl.
Act. necrophorus (Hagan)	+	+	+	+

sl. = slight agglutination.

SEROLOGICAL STUDY

The immediate loss in weight and sometimes the death of rabbits injected with cultures of *Act. pseudo-necrophorus*, usually without evidence of infection, were suggestive of toxemia. An attempt was made to immunize a rabbit by intravenous injections of living culture XXXV at intervals of about 5 days. The serum of this animal was titrated for agglutinins against four pseudo-necrophorus strains as well as against the necrophorus strain. A sample of serum of the same animal collected before immunization was used as a control.

The results of agglutination titration are recorded in Table IV. The homologous strain only was agglutinated in high dilutions of the serum, strain XLIV not at all, and two other strains only slightly. It may be that there is more than one serological group of Act. pseudo-necrophorus. The necrophorus strain was agglutinated only very slightly, if at all, in a serum dilution of 1:10.

After the removal of a sample of serum for control a rabbit was injected intravenously with *Act. necrophorus*, as indicated in Table III. Although, because of the development of infection, the rabbit was not bled at the optimal time for securing a good agglutinating serum it was titrated with the results recorded in Table V. The necrophorus strain was agglutinated in dilutions of serum up to 1:90, whereas the pesudo-necrophorus strains were not agglutinated.

TABLE V

Titration of agglutinins in serum of rabbit immunized against Act. necrophorus (Hagan)

CULTURE	IMMUNE SERUM							CONTROL SERUM			SALT SOLUTION
	1:10	1:30	1:90	1:270	1:810	1:2430	1:7290	1:10	1:30	1:90	
Act. necrophorus (Hagan).....	++	+	+	sl.	sl.	sl.	sl.	sl.	sl.	sl.	sl.
Act. pseudo-necrophorus XLIV..	+	+	+	+	+	+	+	+	+	+	+
Act. pseudo-necrophorus XXXV.	+	sl.	sl.	sl.	sl.	sl.	sl.	sl.	sl.	sl.	sl.
Act. pseudo-necrophorus 8928...	++	+	+	+	+	+	+	++	+	+	+

sl. = slight agglutination.

SUMMARY

From the uteri of women presenting puerperal infection, we have isolated strains of an organism (*Act. pseudo-necrophorus*), closely resembling Act. necrophorus. It differs from the latter in being non-hemolytic when grown in the blood-agar plate and in failing to ferment lactose. The two organisms do not cross-agglutinate in immune sera.

The finding of *Act. pseudo-necrophosus* in three uteri cultured during a series of fifty caesarean sections indicates that it is not uncommonly present, which receives further confirmation from the fact that it was found at autopsy in an additional patient, as well as in the course of the clinical investigation of two other patients who

did not die. In all of six instances the lochia were profuse, thin and foul; in the three women delivered by caesarean section the incision broke down and discharged large quantities of thin, greenish, foul pus; the convalescence of all six patients was prolonged beyond the normal limit and five of the six patients were seriously ill. The history of the cases from which the organism was isolated would indicate that it is not harmless, but it does not appear to be as pathogenic for human beings as is Act. necrophorus for animals, nor is it as pathogenic for laboratory animals injected with it. Because of the strict anaërobic requirements of the organism and its extreme sensitiveness to brief exposure to air, its presence in the human genital tract has probably been overlooked hitherto. It is hoped that further work will reveal the true significance of this organism in human infection.

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THE PHYSICAL RELATION OF CELLS IN TISSUE CULTURES[1,2,3]

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INTRODUCTION

Although the minute architecture of almost every type of cell has received careful study, comparatively little work has been done on the basic interrelationships that obtain between the cells of any tissue. Are the cells of animal tissues independent anatomical and physiological units, or is there a syncytial relation, with numerous protoplasmic bridges connecting adjacent cells? Are cells held together simply by adhesion, or is there a definite organic continuity between them?

The older histologists took for granted the widespread occurrence of intercellular protoplasmic bridges in epithelia and mesenchyme, as well as in smooth, striated, and heart muscle. The appearance of fixed sections would lead to such a conclusion. Not only do cell boundaries often appear incomplete, but there can be seen fine processes or fibrils connecting various cells after "appropriate" fixation and staining. Even with living material, examined *ex vivo* or in tissue culture, cell boundaries seem to be incomplete and a syncytial relation present.

Recent observations on living material suggest strongly that intercellular bridges do not occur nearly so universally as was once believed, if they occur at all. The work of W. H. Lewis (1922, a, b; 1923; 1926), particularly, furnishes strong evidence of the anatomical

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[3] I wish to express my thanks to Dr. and Mrs. W. H. Lewis, in whose laboratory this work was done, and to Dr. Robert Chambers for many helpful suggestions and assistance.

independence of cells in mesenchyme, endothelium, amniotic ectoderm, and heart muscle. This investigator has noted that, both in cultures and in the embryo, cells retain their individuality in spite of their apparent continuity. The extreme variability of adjacent cells in size, shape, and the character of cytoplasmic inclusions (granules, vacuoles, and mitochondria) indicates cellular independence. Cells in cultures readily become isolated and separate without a suggestion of the breaking of connecting bridges. Hogue (1919) reports that, when mesenchyme cells in culture are treated with hypertonic Locke-Lewis solution, the cells withdraw their processes and lose all connection with one another. This indicates that mesenchyme is an adherent reticulum rather than a syncytium. In the case of smooth muscle cells, a similar phenomenon takes place on the addition of a small quantity of glycerine to the culture (M. R. Lewis, 1920). Finally, there appears to be no transfer of material from one cell to another. De Garis (1924) was unable to find any evidence of granules traveling from cell to cell in cultures of fibroblasts. On the contrary, the movements of granules were confined within the limits of a single cell, in spite of the fact that often the cell boundaries were indistinguishable. De Garis states that the fibroblasts further showed their physiological independence by the fact that in cells apparently continuous fluid blebs formed on the surface at distinctly different intervals.

Chambers (1924) has given a unique method of attacking this problem. He has noted that mechanical injury to a cell by means of a microdissection needle will cause coagulation of the nucleus, but that the effect of the injury will not be transmitted to neighboring cells unless there is organic cellular continuity. He concludes that mesenchyme cells, as well as the blastomeres of segmenting echinoderm eggs, are anatomically independent, and states that, in the majority of the cell groups in the metazoan body, there is no evidence of the existence of actual intercellular protoplasmic bridges. In a recent paper, however, Chambers and Renyi (1925) describe protoplasmic bridges as present in stratified squamous epithelium, but as absent in other types of epithelia.

MATERIAL AND METHODS

The observations reported in this paper are microdissection studies on the cells of embryonic chick tissue cultured *in vitro* after the method of Lewis and Lewis. The cultures, for which I am indebted to Dr. Lewis, were from embryos of 4 to 9 days' incubation, grown in a standard Locke-Lewis solution at 39°C. This solution is composed of Locke solution (NaCl 0.9 gram, KCl 0.042 gram, $CaCl_2$ 0.024 gram, $NaHCO_3$ 0.02 gram, H_2O 100 cc.) 85 cc., chicken bouillon 15 cc. and dextrose 0.25 gm. Cultures were examined after incubation of one to nine days, either in the original medium or washed with a dilute solution of neutral red in Locke solution without sodium bicarbonate.

Dissection of cells in the cultures was carried out with microneedles held in a Barber micromanipulator. A coverslip containing a hanging-drop culture was sealed in a moist chamber. Two glass needles, drawn to a very fine point in a microburner and bent at right angles a short distance from the tip, were placed in the manipulator so that their points were first below the hanging-drop suspended in the moist chamber on the microscope stage. The needles were then raised and dissection of cells carried out by means of the manipulator screws, which move the needles accurately in three planes, or by moving the preparation with a mechanical stage, or a combination of both. Continuous observation under oil-immersion was made throughout the operations. Chambers (1922) gives an excellent description of this technique. Dissections were carried out in a warm box kept at approximately 38°C.

The primary purpose of these experiments was to determine whether or not the effect of an injury to one cell in a culture is transmitted to adjacent cells. Chambers (1924a) has shown that the effect of an injury will proceed from cell to cell only if there is cytoplasmic continuity between them. A tear in the surface membrane of one of two mesenchyme daughter-cells, one half hour after the commencement of division, resulted in the coagulation of the nuclei of both cells, in spite of the fact that they were connected by only a very slender strand of cytoplasm. On the other hand, an injury to one of two interkinetic cells, so closely contiguous that no boundary between them could be seen, resulted in the coagulation of the nucleus in the injured cell only.

The hanging-drop culture method of Lewis and Lewis affords a means of obtaining living tissue cells of higher forms in preparations admirably suited for microdissection. The cells are in a fluid medium and are flattened out against the under surface of a coverslip, so that they can be observed with the highest powers of the microscope from above while they are being dissected from below. It is surprising that more work has not been done with a combination of the two techniques. The literature contains no references to any extensive microdissection studies on cells grown in tissue culture. With the exception of a paper by Chambers and Renyi (1925) on teased preparations of epithelia, microdissection studies have been confined to marine ova, protozoa, and the germ cells of certain plants and animals.

MESENCHYME

Cultures of fibroblasts were obtained from subcutaneous tissue, intestine, and heart. The mesenchyme cells migrate out from the explant and form a reticular pattern closely resembling that in the embryo. From their mere appearance one can not ascertain whether the cell processes are actually fused or merely adherent.

Fibroblasts are quite sensitive to mechanical injury, as compared with amebae or sea-urchin eggs. A small tear in the surface membrane will irreversibly injure the cell. The first sign of injury is seen in the nucleus. Immediately after the tear is made, the nuclear membrane, heretofore invisible, stands out sharply. The nucleus swells slightly and then, after a brief interval depending on the extent of the injury, it shrinks and becomes gray and granular. The nucleus of the normal cell appears to be homogeneous, with the exception of one or more nucleoli. There is a marked change in viscosity also. In the normal fibroblast the nucleus is quite fluid but, as coagulation proceeds, it sets to a rigid gel and can be cut into fragments like cheese. If the lesion is sufficiently extensive, the cytoplasm also coagulates and becomes darker and granular. In cells vitally stained with neutral red the changes that take place as a result of injury are more marked. The so-called neutral-red vacuoles and granules lose their color within a few seconds to a minute after a fairly severe tear and the cytoplasm becomes stained a diffuse pink. All degrees of injury can be effected, from immediate and total coagulation, by stabbing

the nucleus violently, to no apparent changes when the needle is cautiously inserted into the cytoplasm.

A large number of fibroblasts in varying degrees of proximity to other cells were injured with more or less severity. In none of the operations (over forty in number) was any coagulation effect visible except in the injured cell. The boundary between an injured and a normal cell can be made out rather easily, since the cytoplasm of the two differs markedly in appearance. Adherent cell processes often show a large overlapping. This can be observed by focusing carefully on the boundary line between a coagulated cell and a normal one. This condition of overlapping had been previously noted by De Garis (1924) from the movement of granules.

An interesting phenomenon in the coagulation of the cell resulting from mechanical injury is that the effect of a mild injury may be reversible. A number of fibroblasts were injured by making a minute tear in the surface membrane. The nuclear membrane became prominent and the nucleus assumed a grayish appearance. The cyoplasm, however, did not coagulate noticeably. In about a half hour the nuclei had resumed their original appearance. If a lesion is sufficiently extensive to cause coagulation of the cytoplasm, the cell cannot recover. This would seem to indicate that the nucleus may show temporary coagulative changes as the result of only a slight injury, but that that coagulation of the cytoplasm is irreversible and a sign of cell death. This observation coincides with those of M. R. Lewis (1923) on gelation of the nucleus and cytoplasm produced by various acids.

The adhesiveness of fibroblasts is markedly altered after injury. Normal cells can be pulled apart only with difficulty. After an injury to the membrane and coagulation of a cell, the adherent processes of neighboring cells often pull away from it and a slight displacement of the coagulated cell with the micro-needle sometimes suffices to separate the adherent processes. Furthermore, cells are less sticky for glass after coagulation than before and may drop away from the under surface of the cover-slip down into the drop. This loss of stickiness upon gelation supports the idea that the adhesive quality of cells is dependent on the surface tension conditions in the surface membrane or film of the cell itself rather than on the presence

of a sticky secretion in the cell or the existence of intercellular protoplasmic bridges. W. H. Lewis (1922, c) describes such a mechanism for the adherence of cells.

A number of other operations were performed on fibroblasts, the results of which are of some interest. The long protruding processes can be pinched off from the rest of the cell without causing any coagulation if care be taken not to rupture the surface membrane. This is a difficult procedure, for the bounding film or membrane is very delicate and easily torn. The neutral-red vacuoles, which appear in the cells of older cultures in large numbers, can be pushed around in the cytoplasm with ease. When the film enclosing a neutral-red vacuole is punctured with a sharp needle, the vacuole is dissipated with explosive violence. This can be accomplished without causing any apparent changes in the rest of the cell. This is what would be expected if one consider the neutral-red vacuoles as degeneration products (Lewis, 1919) and not as a part of the vital architecture of the cell. Several of these vacuoles were carefully removed from cells without injuring the cells greatly. The vacuoles floated freely in the culture medium and maintained their integrity for several minutes, as long as they were in the field. This could not happen unless there were some sort of film enclosing the vacuoles.

It was found that injured and normal cells could be differentiated after fixation and staining. Figure 1 shows a group of fibroblasts from a culture of the skin of a five-day chick embryo. The two injured cells near the center can be distinguished by the fact that their nuclei are stained more deeply and are shrunken and irregular in contour. It will be noted that the cells adjacent to the injured ones are entirely normal in appearance. The culture was fixed one half hour after the operation on the cells.

AMNIOTIC ECTODERM

Ectodermal cells from the chick amnion migrate out on the coverslip and form a continuous sheet or membrane. One can not determine from their appearance whether they are fused or merely adherent. Complete cell boundaries are not distinguishable.

Individual cells in cultures of 2 to 7 days' incubation were injured

in order to determine whether or not the resulting coagulation would spread to adjacent cells. Those within the membrane were injured by vertical stabs, while cells at the periphery were pulled away from the membrane by a lateral displacement of the needle. In the first few attempts coagulation of the nuclei of one or more contiguous cells occurred in about one third of the cases. This was later shown to be due to faulty manipulation rather than to the existence of cytoplasmic continuity.

The cells within the membrane tend to overlap considerably. A stab into a cell, particularly if the needle is inclined at an angle, is apt to produce lesions in adjacent cells. By using only fine vertical needles and taking care to confine the lesion to a single cell, it was found possible to cause coagulation in the operated cell without any changes in neighboring ones. Similarly, improvement in the technique of injuring cells at the edge of the membrane made it possible to confine the effects of a lesion to a single cell. A small tear in the surface membrane on the side distal to the sheet was found to be sufficient to cause gelation of the nucleus without affecting adjacent cells. These ectodermal cells are so firmly adherent to one another that the act of pulling a cell rapidly away from the edge of the sheet produces rupture of the membranes of adherent cells and the subsequent coagulation of their nuclei. If a cell is slowly and gently pulled away from the edge, it can be separated from adhering cells without injuring them. A surer method is to allow the manipulated cell to coagulate completely and then to pull it away from adhering cells. Coagulation, as in the case of fibroblasts, causes the stickiness for other cells to diminish. About thirty ectodermal cells were injured (disregarding the first few faulty attempts) without any evidence of coagulation in cells packed close to them. Observations were prolonged as much as three hours after the coagulation of the injured cell. The relationship that obtains among the ectodermal cells of the amnion is that of adherence rather than organic continuity by fusion or protoplasmic bridges.

The amniotic ectodermal cell membrane is somewhat tougher than that of fibroblasts. The cytoplasm, on the whole, is less viscous and the adhesiveness of the cells for one another appears to be greater. These conditions might possibly explain why ectodermal cells tend

to be rather rounded in shape and to stay together in a sheet. The more viscous and less sticky fibroblasts assume irregular shapes and become separated from one another more readily, thus forming the familiar reticular pattern.

Reversible coagulation of the nucleus after a slight injury occurs in ectodermal cells as well as in fibroblasts, and the nucleus often swells markedly in the first stage of gelation. Neutral-red vacuoles are numerous and quite large in older cultures. In one case an extremely large vacuole, about the size of the nucleus, was carefully punctured with a fine needle. The vacuole was dissipated and the dye quickly diffused throughout the cell, causing it to stand out in clear contrast to circumjacent cells. The color then faded gradually as the dye became resegregated into other vacuoles in the cell. The fact that the neutral red diffused so rapidly through the cytoplasm of the manipulated cell, but did not reach adjacent cells, is a further proof that the surface membrane is entire and that intercellular cytoplasmic continuity does not exist.

Figure 2 shows three injured ectodermal cells on the edge of a membrane. The nuclei appear dark and shrunken, whereas the adjacent cells show no changes.

SMOOTH MUSCLE

Smooth muscle cells are obtained in cultures of amnion. They form a reticulum much like that of fibroblasts. About twenty individual smooth muscle cells were injured, and in no case did coagulative changes take place in any but the injured cell. Smooth-muscle cells are independent anatomical units and are neither fused nor connected by intercellular protoplasmic bridges. Figure 3 shows a group of these cells, several of which were injured by stabbing with a needle. The injured ones can be distinguished from the normal cells by the appearance of their nuclei, which are dark, shrunken and irregular.

ENDODERM

Endodermal cells were obtained from cultures of the stomach and intestine. In migrating, these cells form a membrane typical of epithelial tissues. The overlapping of the cells, as in the case of

amniotic ectoderm, makes it necessary to use care in making a tear in a cell lest other cells become injured in the process. A number of endodermal cells were injured with the needle. Except in a few cases where manipulation was faulty, cells surrounding the coagulated ones showed no signs of injury. Protoplasmic bridges, therefore, evidently do not occur in endodermal tissue.

The loss of adhesiveness of a coagulated cell for other cells can be demonstrated by the following experiment. The needle was inserted vertically into the center of the nucleus of an endodermal cell and left in place for one minute while the cell completely coagulated. The needle was then gently lowered. The coagulated cell, having lost its adhesiveness for surrounding cells, remained impinged on the needle tip and was lifted off, leaving a fenestration in the membrane. This rapid loss of stickiness upon coagulation is an indication of the extent to which the adhesive quality of cells is dependent upon the physical conditions in the surface membrane.

LIVER CELLS

The hepatic cells of the chick grow out in the form of a rather loose sheet. Their physical make up is markedly different from that of the two types of cells described above. The cytoplasm is quite fluid and contains numerous granules. The surface membrane is exceedingly delicate and easily torn. The contour of the cell, as a result of these conditions, is rounded. The adherence of liver cells to one another is very slight. They can easily be pulled apart without injury.

Twenty-five individual hepatic cells were stabbed and in no case was the effect of the injury transmitted to neighboring cells. Figure 4 shows a culture of liver with a sheet of hepatic cells. Injured cells are marked with an →.

ENDOTHELIUM

Endothelial cells from sinusoids appear in cultures of liver. They migrate rather actively and form a loose reticulum. In operation upon these cells there was no evidence of cytoplasmic continuity. Figure 5 shows a group of endothelial cells from a culture of liver.

The cell marked was injured and caused to coagulate. Before the injury, the boundaries between that cell and the one above could not be distinguished. The coarse granular condition of the cytoplasm of the coagulated cell makes it stand out in clear contrast to the uninjured one.

EPITHELIUM FROM THE SKIN

Cultures from the skin of chick embryos contained sheets of squamous epithelial cells. These cells are rather difficult to experiment upon because of their great tendency to overlap. Twelve separate cells were stabbed and in no case did the effect of the injury extend to contiguous cells. This failure to find intercellular protoplasmic bridges in the skin of the chick embryo is interesting in view of the report of Chambers and Renyi (1925) that such bridges do exist in squamous epithelium. Their material included teased preparations of the human epidermis, the stratum lucidum of the human fingertip, the corneal epithelium of the frog, and epithelium from the oesophagus of the mouse.

Figure 6 shows a section of membrane in a two-day culture from the skin of a seven-day chick embryo. The cell marked was injured by a vertical stab into the cytoplasm. The nucleus is darker, shrunken and irregular. No changes are apparent in surrounding cells.

DISCUSSION AND SUMMARY

The results of these observations add another link in the accumulating chain of evidence that fusion of cells in most animal tissues is only apparent and that the intercellular bridges described in many tissues are fixation artifacts. Cells are related to one another merely by adhesion. This is certainly true in the tissues of the chick studied. In view of the conflicting observations of Chambers and Renyi (1925), who describe intercellular bridges in squamous stratified epithelium in other animals, any conclusions drawn from the experiments here reported must be confined, for the present, to the material used.

It would be interesting to know, if protoplasmic bridges do exist in certain tissues, how many cells show coagulative changes after injury to one cell. It has been shown above that the effect of a slight tear in the surface membrane at the distal end of a long cell process spreads

rapidly throughout the cell. Is this coagulation a self-propagated reaction which proceeds as long as there is cytoplasmic continuity? If so, the effects of injury to a single cell in the body would be very widespread. If it can be shown, on the other hand, that only a few neighboring cells are involved, this objection to intercellular bridges becomes invalid.

The evidence reported in this paper for the anatomical independence of cells in the tissues of the chick may be summarized as follows: The effect of a mechanical injury at the surface of a cell is transmitted rapidly throughout the cell, but does not appear to affect adjacent cells. As it coagulates, an injured cell loses its adhesiveness for other cells. This latter fact indicates the importance of surface tension in the natural adhesiveness of cells. The conclusion is drawn that, in the chick, cells of mesenchyme, amniotic ectoderm, smooth muscle, endoderm of the intestine, liver, endothelium, and epithelium of the skin are held together by simple adhesion and not by fusion nor by intercellular protoplasmic bridges.

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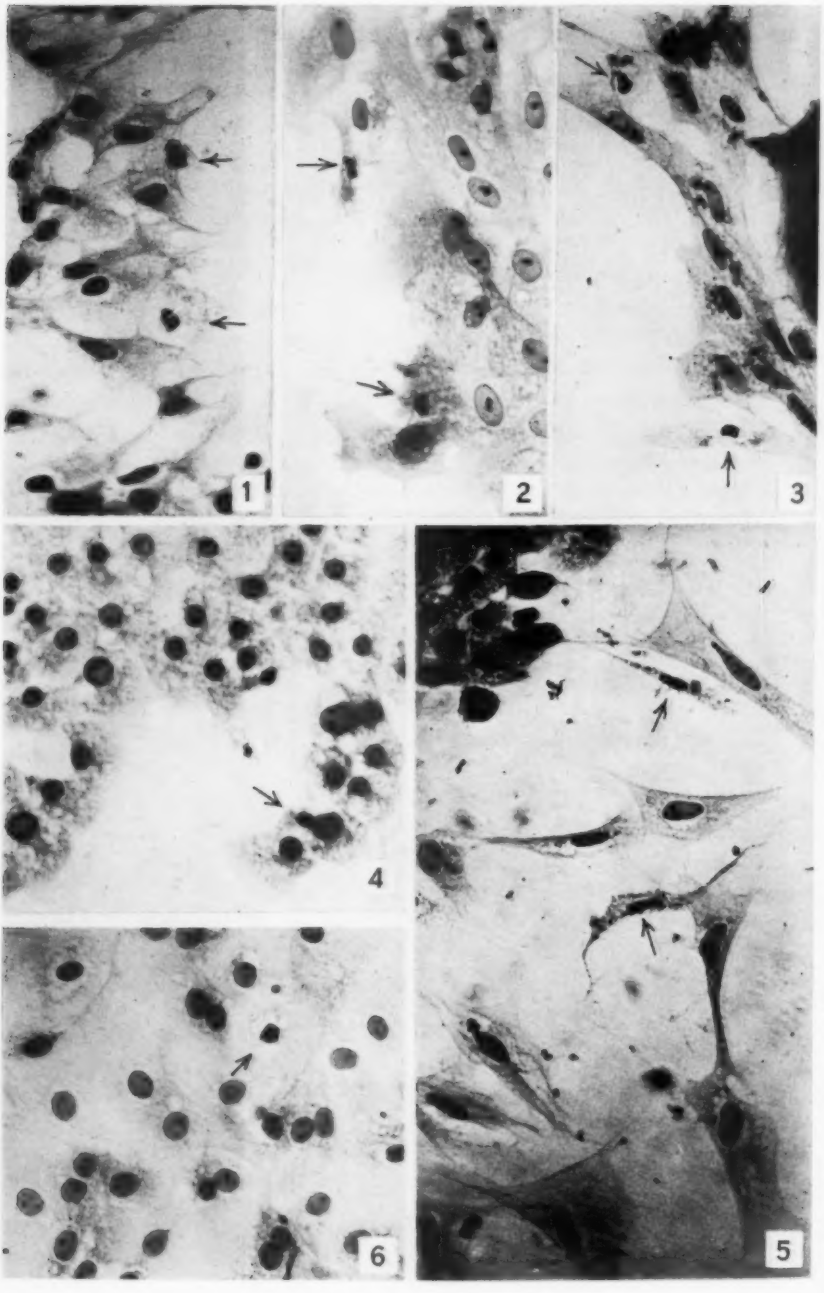
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EXPLANATION OF PLATE 1

All the figures are from cultures fixed in 10 per cent formalin and stained with hematoxylin, orange G, and eosin. The magnification is 450 diameters. Injured cells may be distinguished from normal ones by the fact that their nuclei are pyknotic in appearance and stain more deeply. Arrows point to the injured cells.

1. Mesenchyme. Two-day culture from the skin of a seven-day chick embryo showing reticulum of fibroblasts.
2. Ectoderm. Three-day culture from the amnion of a six-day chick embryo. The two marked cells on the edge of the sheet have been injured.
3. Smooth muscle. Five-day culture from the amnion of a six-day chick embryo.
4. Glandular epithelium. Three-day culture of six-day chick's liver showing hepatic cells.
5. Endothelium. Six-day culture from the liver of a six-day chick embryo showing endothelial cells.
6. Epithelium. Two-day culture from the skin of a seven-day chick embryo.

WINDOWS AND ACTINOMETERS FOR HELIOTHERAPY[1]

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The curative effects of ultra-violet light in cases of rickets and tuberculosis have been demonstrated so amply that no further arguments need be adduced in support of this mode of treatment. The sources of ultra-violet light most generally employed are the sun and the quartz-mercury arc. The latter is quite expensive, while the former can be used effectively, for infants at least, only during the warm seasons. To make sunlight available also during the cold seasons, the suggestion to use windows made of materials transparent to ultraviolet light is obvious. While sheets of fused quartz and of new glasses are now available, they are all more or less expensive and, therefore, not within the reach of the poor who, after all, are the ones most in need of heliotherapy. It is the purpose of the present paper to present a type of window which is cheap, portable and efficient—and also, to describe an actinometer for measuring the intensities of curative radiations.

WINDOWS

In connection with a study of the transparency of a variety of cellulose compounds, the spectrograms shown in Fig. 1 were obtained., Since the therapeutic region for sunlight lies between 3200 and 2900 A.U. it is evident from strip 2 that window-glass is almost useless. The other materials, as shown by strips 3 to 6, inclusive, develop pronounced opacity in the region around 3000 A.U. By far the most transparent material of this type is cellophane—as shown by strip 7. This material is regenerated cellulose and is used widely in the manufacture of artificial silk and transparent wrappings for delicacies. The actual transmission curve for this material is presented in fig. 2 and it is shown that, in the most effective region

[1] Received for publication, Jan. 12, 1927.

(around 3000 A.U.) the transmission is still between 65 and 70 per cent.

Cellophane is manufactured in large sheets and is, therefore, well adapted for the manufacture of windows. It is true that ordinary cellophane, when wet, becomes limp, and when dry, becomes brittle. These defects have been overcome in the recent development of a moisture-proof cellophane,[2] which is but little less transparent than the original material and which is unaffected by changes in atmospheric humidity.

In addition to its greater transparency to ultra-violet light, cellophane has a further advantage over other cellulose products in that

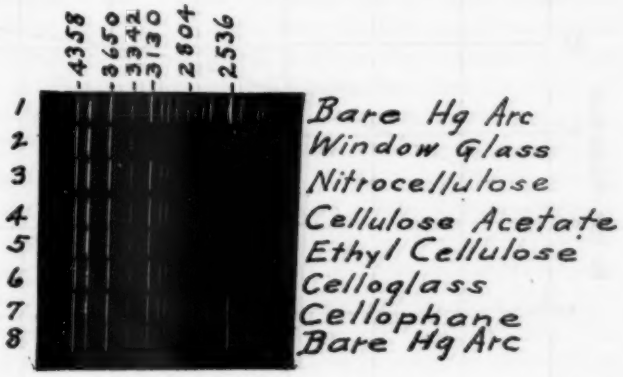
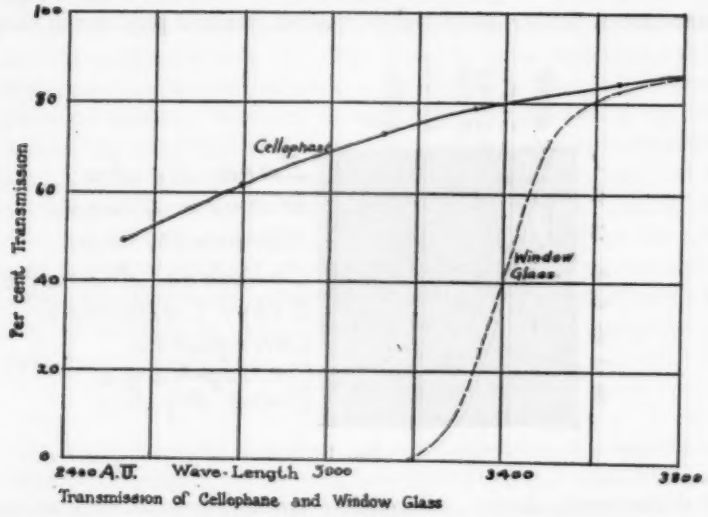


FIG. 1

it yellows very slowly. All cellulose products become more or less yellow upon exposure to ultra-violet light and, in consequence, sacrifice more or less of their ultraviolet transparency. A simultaneous exposure of the five cellulose materials, shown in Fig. 1 to the radiations of a quartz mercury arc for 10 hours showed that, while nitrocellulose had turned a yellowish-brown, the change in cellophane was just barely detectable. The other cellulose compounds lay between these extremes—the yellowing being, in general, dependent upon the degree of absorption of the material for ultra-violet rays.

[2] Detailed information concerning cellophane windows may be obtained from the DuPont Cellophane Company, Buffalo, N. Y.

Another interesting property displayed by cellophane is that of double refraction. Between crossed Nicol prisms, cellophane is found to possess two directions of vibration, respectively parallel and perpendicular to the direction in which the material has passed over rollers while being manufactured. The double refraction is retained even though the cellophane has become quite limp as a result of soaking in water. Since cellophane is procurable in large sheets and in a variety of thicknesses it may be used for the production of quarter and half-wave plates of large area.



Transmission of Cellophane and Window Glass

FIG. 2

ACTINOMETERS

While it is recognized that there is a rather wide variation in the sensitiveness to ultra-violet light of the skin of various individuals—in consequence of which variation no fixed light dosage may be prescribed—it is nevertheless contended that, for purposes of record and correlation of results, a reproducible instrument for the determination of intensity and dosage is desirable. The first demand to be made upon such an instrument is that it shall respond only to the therapeuti-

cally active radiations, and to these, in the measure in which they effect the cure of the ailment in question. The first step is, therefore, to determine the relative effectiveness of the various spectral regions in bringing about curative effects. While no such measurements for the treatment of rickets and of tuberculosis are at hand, there is an excellent curve by Hausser and Vahle[3] which reveals the relation of the reciprocals of minimum dosage for erythema, i.e., "sensitivity" *and* the wave-length of the stimulating radiation. As shown in Fig. 4, curve D, this curve has a very sharp maximum near 2975 A.U.—the region of maximal effectiveness for erythema. While the falling off toward shorter wave-lengths is, without doubt, to be attributed in part to the increasing opacity of the epidermis, the very abrupt drop toward longer wave-lengths must be associated with the response of the living cells to ultra-violet radiations. This contention is supported by the well-known facts that the effect of ultra-violet light upon the coagulation of albumen as well as its bactericidal action have approximately, the same "long-wave-length-limit" as has the curve for erythema. While, as previously stated, no similar curve exists for the treatment of rickets, it seems probable that such a curve will have a form similar to that for erythema. Hess[4] has shown by means of light filters that the most effective rays for the therapy of rickets lie at wave-lengths shorter than 3200 A.u. Again, since in the treatment of rickets as well as of erythema, the shielding effect of the epidermis will also be in evidence, the relative effectiveness of the shorter radiations will again result in a falling off of the curve.

Of the various actinometers for light therapy, the only one which approaches the ideal, previously mentioned, is that involving the use of lithopone. Subsequently to the writer's[5] determination of the spectral sensitivity of the darkening of lithopone, he suggested the use of this material for therapeutic actinometers and developed the

[3] Hausser and Vahle: *Strahlentherapie*, 13, p. 41 (1922).

[4] Hess: *Jour. Am. Med. Assn.*, **80**: No. 10, 687 (1923).

[5] Pfund: *Proc. Am. Soc. for Testing Materials*, 1923. A more accurate determination has since been carried out by Dr. F. G. Bricwedde: J. H. U. Dissertation (1925).

testing device embodied in the work of Clark.[6] The latter showed that the darkening of lithopone was, indeed, a fairly accurate measure of light dosage for erythema for sources as widely different in intensity distribution as the quartz mercury arc and the iron arc. A further contribution resulting from her work was the suggestion of a unit for dosage, *i.e.*, the "lithopane unit." This is, by definition, the total quantity of ultra-violet light (intensity $\times$ time) required to cause an initially white water paste of lithopane, in contact with, and exposed through, a quartz plate, to darken until the coefficient of diffuse reflection has dropped to the absolute value of 50 per cent.

While the manipulation with lithopone is very simple and satisfactory if the same powerful source of ultra-violet light be employed throughout, it suffers from a number of drawbacks:

(1) The response to feeble radiations is quite uncertain.

(2) The maximum of response lies at too great a wave-length (3130 A.u.) and extends too far into the region of longer wave-lengths (see Fig. 4).

(3) The time consumed in carrying out preliminary operations and in making actual measurements is too great.

In the instrument about to be described these defects have been largely overcome.

From the very outset it was decided to make use of a fluorescent glass, in order that a complete separation between the fluorescent light and the visible light from the source might be effected. In this respect a fluorescent glass has a great advantage over powdered materials, such as zinc sulfide or barium platinocyanide, whose fluorescent radiations are greatly masked by the scattered visible light. As a first step, photometric measurements on the intensity of visible fluorescence, excited by the various mercury lines, were made. Since the energy carried by each mercury line had been measured with a vacuum thermopile, it was possible to determine the intensity of fluorescence for each mercury line "at constant energy." Having thus eliminated the effect of variation of intensity among the various mercury lines, it was possible to plot a "sensitivity curve for visible fluorescence."

[6] Clark, J. H.: *Am. Jour. Physiology*, 1924, lxix, 200.

The fluorescent glass actually used was uranium glass whose sensitivity variations are shown in curve A, Fig. 3. The excitation of fluorescence by visible radiations was eliminated by passing the light through a thin-walled glass bulb, 5 cm. in diameter, filled with bromine vapor—the transmission curve for the combination of bulb and bromine being shown in curve B, Fig. 3. Since the curve obtained by plotting the products of corresponding ordinates of curves A and B was still quite different from the Hausser and Vahle curve (Fig. 4), an endeavor was made to bring about a closer agreement by covering half of the surface of the uranium glass with a plate of clear glass,

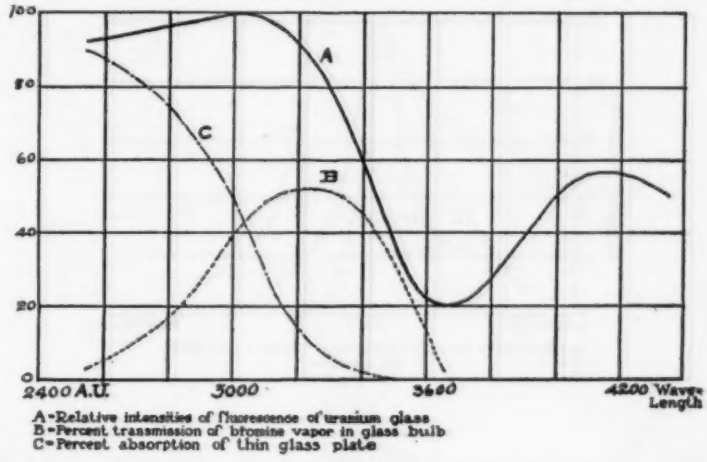


FIG. 3

and the other half with a plate of crystalline quartz. If the entire system be now illuminated by radiations from a mercury or iron arc, it is evident that the *difference* in brightness of fluorescence from the quartz-covered and from the glass-covered halves of the uranium glass will be caused by that portion of the ultra-violet radiation which does not get through the clear glass. This arrangement is essentially equivalent to a filter whose *transmission* curve is the *absorption* curve for the glass (curve C, Fig. 3). Finally, as a result of multiplying together the values of the corresponding ordinates of curves A, B

and C, the curve E, Fig. 4, was obtained. For the sake of comparison, the Hausser and Vahle curve (D) and the curve for lithopone (F) are inserted. While the agreement between curves D and E is not so good as might be desired, it is felt that, in view of the number of variables[7] available for modifying curve E, it will always be possible to effect a closer agreement as soon as the true curve for the treatment of rickets shall have been established.

The above concepts have been embodied in an instrument which is shown in Fig. 5. Radiations from the source of ultra-violet light A, after passing through the bromine bulb (R), fall upon the glass

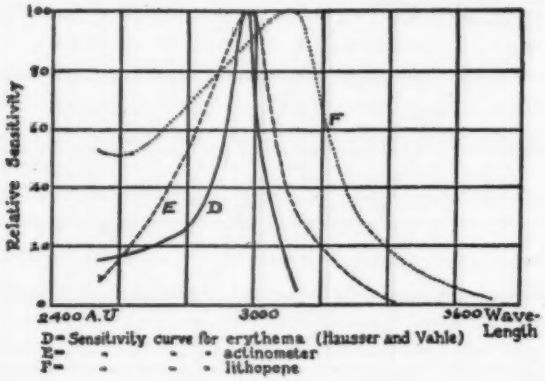


FIG. 4

plate (G) and the quartz plate (Q) and are finally received by the plate of uranium glass (U) which is cemented with Canada balsam to the prism of clear glass (P). Since the apparent brightness of a fluorescent surface increases greatly as grazing angles are approached (owing to a failure of Lambert's law), the fluorescent radiation is taken out in the direction indicated and is allowed to fall on the biprism (B), while the visible radiations pass on and are ultimately absorbed by the blackened faces of the prism (P). As seen through

[7] In addition to the devices employed, the possibility of using silver films, chlorine, nickel-ammonium sulfate and a number of aniline dyes with transmission bands near 3000 A.U. is suggested.

the eye-piece (E), the bi-prism presents a field such as is shown in the insert (Fig. 5), the bright area (Q) being that portion of the uranium glass whose fluorescence is due to radiations transmitted by quartz, while the darker area (G) is the corresponding portion back of the glass. Since we are interested in the *difference* between these two brightnesses, it is only necessary to add light to the darker area (G) to establish a photometric match. This is accomplished by means of a small incandescent lamp (I) whose radiations are

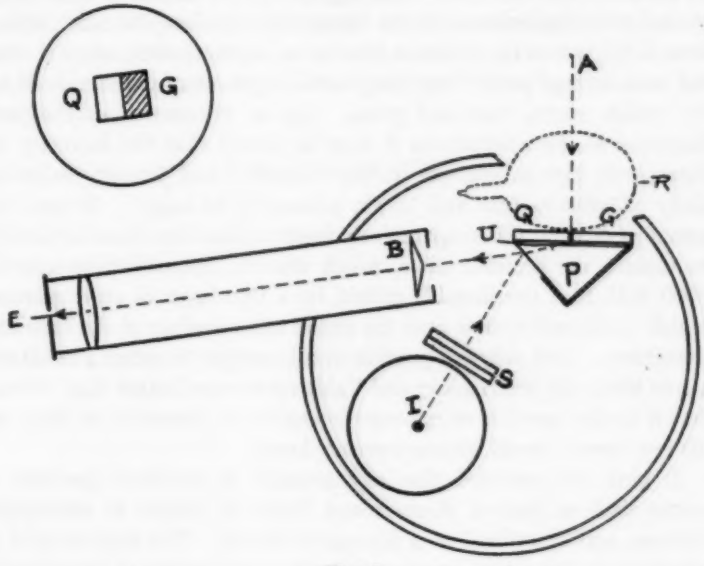


FIG. 5

passed, in succession, through a diffusing screen and a green filter (S) which renders the transmitted light of the same color as that of the fluorescent light. The green light from (I) is next reflected from the prism face (P) and is superposed on the darker field (G) alone. By varying the current flowing through (I), a photometric match is readily established.

The instrument is calibrated by means of a rotating sector, transmitting known fractions of the incident radiation (A). For each

setting of the sector, a photometric match is established by varying the current flowing through the lamp (I). Readings are recorded as taken off an ammeter, connected in series with (I) or a voltmeter shunted around (I). For such calibration, a distance of 50 cm. between the source of ultra-violet light and the actinometer has been found very satisfactory.

Without going into minute details, it may be stated that it has been found best to use the apparatus as described when measurements on intense sources of ultra-violet light are to be made. Particularly in case of measurements on the therapeutic efficiency of solar radiations is the use of the bromine filter to be recommended, since it cuts out such a large part of the long wave-length ultra-violet as well as the visible violet, blue and green. (As an illustration of the performance of the actinometer it may be stated that the intensity of therapeutic rays in sunlight during December and January is essentially nil while in June and July it is found to be large). In case the source of radiation is comparatively feeble, it has been found desirable to replace the bromine filter, which absorbs the radiations around 3000 A.U. to a considerable extent, by a thin layer of clear gelatine which is allowed to flow over the entire outer surface of the uranium glass plate. Not only is it possible in this manner to obtain a resultant curve which fits the Hausser and Vahle curve even better than before, but it is also possible to measure intensities at distances as great as 20 feet from a small quartz mercury lamp.

It must be conceded that any attempt to duplicate precisely a curve such as that of Hausser and Vahle by means of absorption screens, and other devices is doomed to failure. The only method of precision is that which involves a quartz spectroscope of considerable dispersion. If a metallic template be made of the form of the curve to be copied, *i.e.*, if the metal be removed in the area between the base line and the curve, and if this template be mounted in the focal plane of the spectroscope, the relative amounts of the various spectral regions transmitted by the template can be made to follow the curve to be copied, to a high degree of accuracy. Subsequent recombination and intensity measurement of the radiations transmitted by the template can be effected readily.

For purposes of defining dosage measurements and of correlating

results in heliotherapy, it is suggested that, after a spectral curve of response for the treatment of rickets (similar to the Hausser and Vahle curve for erythema) shall have been established and that, after the curve of response of the actinometer shall have been modified to fit the rickets curve, measurements on light intensity be carried out with an instrument embodying the principles outlined. For standardization of all instruments and methods the iron arc, as developed by the writer for use in spectroscopy,[8] is suggested. This arc, when operated at 5 amperes and a 30 volt drop across the terminals yields a readily reproducible source of ultra-violet light, which bears the same relation to ultra-violet actinometry that the standard candle does to visual photometry. Work on such methods of standardization is in progress.

[8] Pfund: *Astroph. Jour.*, Vol. 27, p. 296 (1908).

THE AMYLASE-ACCELERATOR AND ANTI-TRYPSIN OF NORMAL HUMAN SERUM[1]

CHARLES O'DONOVAN, JR., AND WILBURT C. DAVISON

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Pozerski (1), Wohlgemuth (2), and Crohn and Epstein (3) noted that the addition of serum to preparations containing amylase increased their starch-digesting power. Two of these observers (1, 3) tested the effect of heat and reported that keeping serum at temperatures as high as 100°C. for 30 minutes did not diminish its amylase-accelerating activity; the third did not study this phase of the question. The present experiments are reported, first, because the presence of an amylase-accelerator in normal serum has been confirmed by a different method (viscometric (4)), the earlier observers having used saccharogenic (1) and iodine color procedures (2, 3); second, because, in contradiction to the previous reports, heating the serum to 58°C. for 30 minutes produced a marked reduction in the activity of the amylase-accelerator; and third, because these studies illustrate the contrast between the effect of serum on amylase with that on trypsin.

1. *Amylase-accelerator.* Although amylase (diastase) can be demonstrated in the serum of normal and diseased individuals by methods which measure saccharogenic activity, no starch-liquefying power is manifested in tests which determine viscometric changes (5). However, in spite of this absence of starch-liquefying activity, the addition of serum to pancreatic preparations increases their ability to liquefy starch, as compared with controls without serum (Chart 1 and Table I). Serum also increases the activity of the starch-liquefying power of the duodenal contents of infants. Heating the serum to 58°C. reduces its power to accelerate amylolytic activity. This phenomenon was observed in several determinations made at different hydro-

[1] Received for publication January 28, 1927.

gen-ion reactions. The serum of a nephritic patient had the same effect. A typical protocol is given below:

Experiment 1. Ten cubic centimeters of boiled and autoclaved 4 per cent Lintner starch dissolved in $\frac{M}{20}$ phosphate mixture at pH 7.0 were placed in each

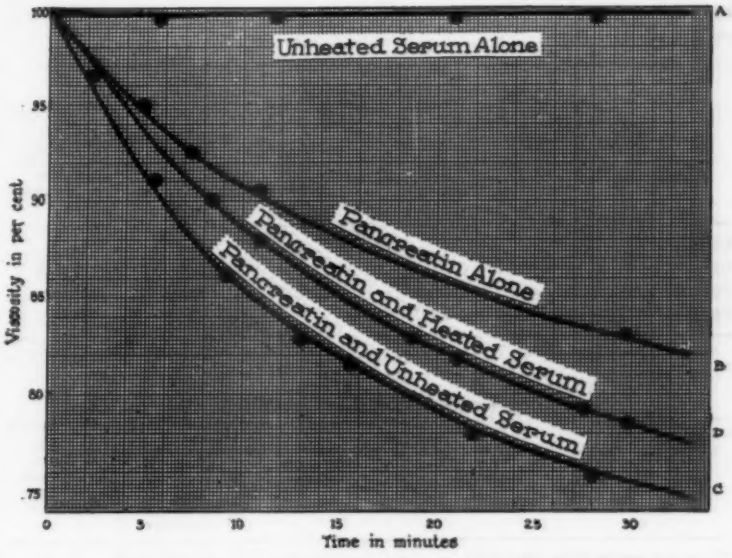

CHART 1. AMYLASE-ACCELERATING ACTION OF NORMAL SERUM

Curve A. Effect of unheated normal serum on the viscosity of a starch solution.

Curve B. Effect of pancreatin on the viscosity of a starch solution.

Curve C. Effect of a mixture of unheated normal serum and pancreatin on the viscosity of a starch solution.

Curve D. Effect of a mixture of heated normal serum and pancreatin on the viscosity of a starch solution.

of four large Ostwald viscosimeters immersed in a glass water-bath at 34°C. The time required for 5 cc. of the solution to flow through the capillary tube of the viscosimeter was determined several times with a stop-watch. This time value will hereinafter be designated as the "viscosity" of the solution. The following four mixtures were made, kept at room temperature for 30 minutes

and then added to one of the four viscosimeters: (A) 0.3 cc. of unheated pooled normal serum and 0.1 cc. of distilled water; (B) 0.3 cc. of distilled water and 0.1 cc. of 0.2 per cent pancreatin (Holadin suspended in distilled water and then filtered); (C) 0.3 cc. of unheated pooled normal human serum and 0.1 cc. of 0.2 per cent pancreatin and (D) 0.3 cc. of pooled normal human serum, previously heated at 58°C. for 30 minutes, and 0.1 cc. of 0.2 per cent pancreatin. The contents of each viscosimeter were then mixed and the time noted. The viscosity of these mixtures was determined at intervals of three or more minutes for a period of one hour.

TABLE I

Amylase-accelerating action of normal serum

MIXTURE	TIME (IN MINUTES) REQUIRED FOR 20 PER CENT REDUCTION OF THE INITIAL VISCOSITY	UNITS OF AMYLASE PER GRAM OF PANCREATIN
Unheated serum alone (curve A)	∞	0
Pancreatin alone (curve B)	40	7,500
Pancreatin and unheated serum (curve C)	18.5	16,200
Pancreatin and heated serum (curve D)	25	12,000

TABLE II

Anti-tryptic action of normal serum

MIXTURE	TIME (IN MINUTES) REQUIRED FOR 10 PER CENT REDUCTION OF THE INITIAL VISCOSITY	UNITS OF TRYPSIN PER GRAM OF PANCREATIN
Unheated serum alone (curve E)	∞	0
Pancreatin alone (curve F)	9	520
Pancreatin and unheated serum (curve G)	∞	0
Pancreatin and heated serum (curve H)	19	248

In Chart 1, the results have been plotted on coördinate paper, the abscissae representing the elapsed time between observations, and the ordinates the observed viscosities expressed as percentages of the initial viscosity; the initial viscosity which was measured in seconds being expressed as 100 per cent (4). Unheated serum did not affect the viscosity of the starch solution (curve A), but accelerated the starch-liquefying activity of pancreatic solution (curve C) as compared with the control without serum (curve B). The heating of serum to 58°C. for 30 minutes reduced its amylase-accelerating effect (curve D).

This effect of serum on the starch-liquefying activity of pancreatin may also be expressed mathematically (Table I). One unit of amylase is that amount which requires 60 minutes to reduce the initial viscosity 20 per cent. In curve B, for example, the initial viscosity was reduced 20 per cent in 40 minutes and, therefore, 0.1 cc. of 0.2 per cent pancreatin contained $\frac{60}{40}$ or 1.5 units, and 1 gram of pancreatin con-

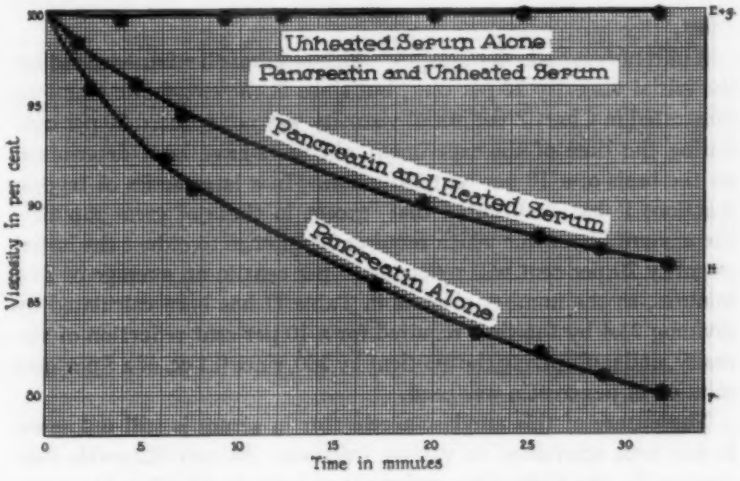

CHART 2. ANTI-TRYPTIC ACTION OF NORMAL SERUM

Curve E. Effect of unheated normal serum on the viscosity of gelatin.

Curve F. Effect of pancreatin on the viscosity of gelatin.

Curve G. Effect of a mixture of unheated normal serum and pancreatin on the viscosity of gelatin.

Curve H. Effect of a mixture of heated normal serum and pancreatin on the viscosity of gelatin.

tained 1.5 × 5000, or 7,500 units. As may be seen in Table I, the addition of unheated human serum more than doubled the starch-liquefying activity of pancreatin, while heating the serum to 58°C. for 30 minutes reduced its amylase-accelerating effect by 50 per cent.

2. *Anti-trypsin.* The experiments were similar to those described for amylase except that 3 per cent gelatin at pH 7.4 (6) was substituted

for the 4 per cent starch solution, and that the pancreatin solution used was 5 per cent instead of 0.2 per cent. As depicted in Chart 2 and Table II, the addition of unheated normal human serum to pancreatin completely inhibited its tryptic activity (curve G), while serum heated to 58°C. reduced it 50 per cent (curve H). Normal serum did not affect the viscosity of gelatin (curve E). Normal serum also inhibited the gelatin-liquefying activity of specimens of duodenal contents, and heating the serum reduced this anti-tryptic effect.

A unit of trypsin is that amount which requires 60 minutes to reduce the initial viscosity 20 per cent, but since in the enzymatic reactions illustrated in Chart 2 the initial viscosity was not reduced 20 per cent during the time of the experiment, the units have been determined on the basis of a 10 per cent reduction. In a large series of trypsin reactions it has been noted that, except in those of great rapidity, the amount of enzyme which requires 60 minutes to reduce the initial viscosity 20 per cent will reduce it 10 per cent in an average of 23.4 minutes, so the number of units in Table II has been calculated by dividing 23.4 by the time required for a 10 per cent reduction of viscosity, and multiplying the quotient by 200, since 0.1 cc. of a 5 per cent solution of pancreatin was used.

The nature of this amylase-accelerator in serum is still unknown. It has been attributed to various fractions: the non-dialysable substances (1), the dialysable portion (3) and the lipoids (2). However, the evidence is contradictory, for Pozerski (1) found that the dialysis of serum had no effect on its amylase-accelerating power, while Crohn and Epstein (3) described a reduction after dialysis.

CONCLUSION

The blood serum of normal individuals contains an amylase-accelerator as well as anti-trypsin. Heating the serum to 58°C. for 30 minutes reduces the amount of amylase-accelerator and of anti-trypsin about 50 per cent.

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THE INFLUENCE OF THE ADMINISTRATION OF COD-LIVER OIL TO THE MOTHER ON THE DEVELOPMENT OF RICKETS IN THE INFANT[1]

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The question of how much protection against rickets can be conferred on an infant through factors which affect the mother, either in the form of an inherited antirachitic reserve present at birth or as a prophylactic substance (vitamin D) transmitted through her breast milk, is of importance in relation to etiology and prophylaxis. The curative and prophylactic action of cod-liver oil and other foods which contain vitamin D, as well as the similar effect of ultra-violet light and sunlight, is now generally conceded when the therapy is applied directly to the infant. It is natural to suspect that both of these factors may exert an influence on the mother which she can carry over indirectly to the infant.

In 1924, Luce (1) at the Lister Institute studied the antirachitic effect on rats of milk obtained from a cow kept under varying conditions of feeding and sunlight. She concluded that feed was more important than light in increasing the antirachitic potency of the milk but said that a maximal effect was not reached until the cow was placed under conditions of maximal light and optimal diet. Steenbock, Hart, Hoppert and Black (2), in 1925, showed that the antirachitic potency of goat's milk as determined by the amount necessary to induce healing in rachitic rats was definitely increased after the animal had been exposed to radiations from the quartz mercury vapor arc. Recently, Hess, Weinstock and Sherman (3) have described an experiment in which they were able to demonstrate the development of antirachitic substances in the breast milk of a woman who had been exposed for a month to light from the mercury vapor lamp. Prior to the irradiation of the mother, the milk had no effect on rachitic rats but

[1] Received for publication, February 14, 1927.

subsequently marked evidences of healing could be demonstrated. It was also shown that this effect was not due to change in the phosphorus content of the milk.

Other investigators have shown that factors present in the maternal diet may influence the development of rickets in the young. In 1923 Korenchevsky and Carr (4) fed rats on a high fat-soluble vitamin diet throughout the periods of pregnancy and lactation and both and noted the effect on the offspring when later they were placed on rickets-producing diets. They concluded (*a*) that, when the mother is fed during pregnancy or lactation on a diet deficient in fat-soluble factor or calcium, there is a marked increase in the disorders of general nutrition and rachitic changes in the skeleton of the offspring kept after weaning on a diet deficient in fat-soluble factor or calcium; and conversely (*b*) that when the mother is fed during pregnancy on a diet rich in fat-soluble factor and calcium there is a marked decrease in the disorders of general nutrition and rachitic changes in the skeleton of the offspring kept after weaning on a -A diet, even when the mother was also kept on the same insufficient diet during lactation; and (*c*) that feeding the mother during lactation on a diet rich in fat-soluble factor and calcium hinders, for a considerable time, the disorganization of general nutrition and the development of obvious rickets in the offspring kept on a diet deficient in fat-soluble factor. In 1924, in a series of similarly conducted experiments, Hess and Weinstock (5) were unable to prevent the development of rickets in the offspring when an unquestionably adequate diet was fed the mother rats during either the periods of pregnancy or lactation, but noted that when the same diet was continued through both pregnancy and lactation the degree of rickets was somewhat mitigated. From a series of earlier experiments (6) these authors had concluded that cod-liver oil, when given to the mother during pregnancy, cannot be relied on to protect the offspring from rickets and that the active principle of the oil is not excreted in the milk in adequate amounts. In 1925 Grant and Goetsch (7) observed from experiments on rats in which mothers and litters were kept in darkness on a diet partially deficient in the artirachitic vitamin that, although first litters developed normally, the development of subsequent litters was retarded and that marked and severe rickets occurred in the young of the fourth and fifth litters. Further-

more, in control experiments, when extra vitamin was added to the same diet, third and fourth litters developed normally. It is unfortunate that in these excellent experiments no histological studies were made.

Recently McCollum, Simmonds, Becker and Shipley (8) have published the results of a series of carefully designed experiments. They call attention to the fact that Korenchevsky and Carr did not distinguish between vitamin A and the antirachitic vitamin D and that the diets employed by them were deficient in both of these factors. In their experiments care was taken to distinguish between these two vitamins and from them they conclude that when female rats are fed cod-liver oil before mating, during pregnancy and during the first two weeks of lactation, their young are protected to a certain degree against rickets when they are later subjected to a rickets-producing diet. They believe that their experiments demonstrate that the vitamin D of cod-liver oil does pass into the mother's milk.

In summing up the results of these investigations it is apparent that the observers agree that the diet of the mother rat may have some influence on the development of rickets in the young, but are somewhat at variance as to the amount of protection which may be so transferred. Up to the present there have been no direct observations on the human infant to show that its resistance to rickets has been influenced by maternal factors. Hess and Weinstock attempted to gather information on this point by administering cod-liver oil to pregnant mothers during the last two months of pregnancy but were unable to prevent the subsequent development of rickets by so doing. The frequency of rickets in breast-fed infants, especially those of Italian and negro parentage, is well known. Our conclusions concerning the etiology of the disease in these cases, however, must be deduced from our knowledge of the disease in experimental animals. On the whole, the similarity of the disease in infants to that experimentally produced in animals is so close that in all probability the conclusions are justified. Nevertheless, they are of such importance that direct verification in the human has seemed desirable.

In the present investigation the problem was approached from the statistical point of view. Different groups of mothers with nursing infants were fed varying amounts of cod-liver oil during the period of

lactation and the incidence and degree of rickets in the different groups compared.

PLAN OF SURVEY

At the outset it was determined to limit the survey entirely to negroes, as the incidence of rickets in breast-fed infants of this race is extremely high when no prophylactic regime has been instituted. In Baltimore the negroes constitute a large percentage of the patients attending the free dispensaries and they are, taken as a whole, more coöperative in carrying out the instructions of physicians than the dispensary class of white people. Through the kindness of Dr. J. Whitridge Williams I was permitted to begin the study on the colored obstetrical ward of the Johns Hopkins Hospital. Every morning the ward was visited and the names and addresses taken of all women who had been admitted for delivery during the previous day. Out of every four women one was kept as a control; for the remaining three an order was left with the nurse for the administration of a teaspoonful (5 cc.) of cod-liver oil three times a day.[2] No patients were enrolled for study whose mothers had not been delivered in the hospital. In general these were young mothers keenly interested in the welfare of first babies and it was felt that during the short stay in the hospital (in most cases two weeks) they might become somewhat accustomed to the none too palatable cod-liver oil. Before discharge, the babies were given a routine physical examination and each mother was talked to individually in an effort to enlist her coöperation. She was given an eight ounce bottle of the oil to take with her on leaving the hospital and told how to obtain more when it was gone. Lastly she was impressed with the advantage to her baby of breast feeding.

Immediately upon discharge the names of all patients enrolled were sent to one of the agencies which conduct well baby clinics in

[2] This was the prescribed dosage throughout the survey. Although it was felt that the dose was very small when compared with the amount per kilogram of body-weight given to infants, I did not feel we could expect the mothers to coöperate if they were asked to take more. As it turned out, even this dose was too large for the majority of the women and it required continual encouragement to persuade even a small number to be faithful in taking the oil throughout the period of observation.

Baltimore.[3] Within a few days a nurse from the agency visited the mother, encouraged her to be regular about taking the oil, told her that more could be obtained either from her or at the clinic, and invited her to bring her baby regularly to the clinic for observation. The mothers of control patients were merely invited to the clinic and the desirability of breast feeding stressed. To them no mention was made of cod-liver oil. At the clinic the histories of these patients were specially marked to notify the attending pediatrician that cod-liver oil was not to be prescribed for the baby. When, as it happened in several instances, he thought that an infant was developing such a degree of rickets that continuation of the experiment was inadvisable, the infant was referred to the hospital, where roentgenograms of the extremities and blood analyses were obtained and cod-liver oil prescribed for the baby. When a mother failed to come regularly with her baby to the welfare clinic, she was visited in her home by the nurse at fairly frequent intervals. The amount of cod-liver oil consumed by each mother was estimated by counting the number of bottles necessary to keep her supplied.

When the infants were approximately six months old, a form letter was sent out from the hospital, requesting the mother to bring her baby to the dispensary for a special examination for rickets. At the time of this examination the infants were weighed, gross evidences of rickets noted, roentgenograms of the extremities secured showing the distal ends of both radii and ulnae and of both tibiae and fibulae, and a specimen of blood obtained for analysis for the serum content of calcium and inorganic phosphorus. Also, the mother was questioned carefully concerning the use of supplementary or complementary feedings and as to whether the baby had been given any cod-liver oil. Only those babies who had been exclusively breast fed were included in the study. The results of these examinations were communicated

[3] In Baltimore there are two agencies conducting welfare clinics for examination of normal infants and advice to mothers concerning their care and diets—the Bureau of Child Hygiene of the City Department of Health and the Babies' Milk Fund Association. The 28 wards of the city are assigned to one or the other of these organizations. They conduct 46 welfare clinics, the operation of which is facilitated by a corps of 29 nurses and a dozen or more physicians. It was only through the coöperation of these excellently organized agencies that adequate follow-up work in the present series of cases was possible.

as soon as possible to the welfare clinics, so that cod-liver oil could be prescribed for the baby.

The cod-liver oil used was of good Newfoundland variety and was all from the same lot. It was donated by a reputable manufacturer, who had tested its antirachitic potency by the usual line test in rachitic rats. As a further verification the test was performed by Dr. P. G. Shipley of the Johns Hopkins Pediatric Department and found positive.

METHODS OF INTERPRETING ROENTGENOGRAPHIC FINDINGS

In order that the roentgenograms might furnish a mensurable figure indicating the severity and duration of rickets when present and suitable for the preparation of averages in each group, a special but simple method of interpretation was employed. The number 0 was given to those films in which there could be no doubt that rickets did not exist, that is, when the ends of the shafts were sharply defined, slightly convex, and the periosteal border either sharply marked or somewhat rounded. The number 1 was given to films which in the past we have regarded as showing slight evidence of rickets, that is, to roentgenograms in which the ends of the shafts appeared broken or slightly irregular in one or more places and the corners showed small lips of calcified bone projecting toward the epiphysis. In view of the finding by Eliot (9) and Wilson (10) of such slight changes in infants in whom antirachitic therapy had been prolonged and adequate, it now seems doubtful whether they should be regarded as manifestations of disease. The number 2 was assigned to roentgenograms when the ends of the shafts showed a furring over their entire length producing an early but definite fray, and no longer appeared convex, and when the projections at the periosteal borders were moderately advanced. In this group, rickets as a disease was considered definitely to be present. The number 3 was given to a film when the rachitic changes were regarded as advanced, that is, when there was well marked fraying and concavity at the ends of the shafts and the shadow cast by the cortex showed evidences of osteoporosis. Before grading a film, the distal ends of the radii, ulnae, fibulae, and tibiae were all examined and an average impression obtained. In general, however, it was felt that the most satisfactory impression could be secured from the changes at the distal end of the ulna. The films were read by two observers. In most cases

there was complete agreement as to the number to be assigned to the picture. In a few cases where doubt existed as to the proper grading, the average figure between the two doubtful grades was assigned, thus, 0.5, 1.5, or 2.5.

INTERPRETATION OF BLOOD SERUM FINDINGS[4]

From their analyses of the blood serum in normal and rachitic infants, Howland and Kramer (11) concluded, in 1922, that when the product of the calcium and inorganic phosphorus concentrations expressed in milligrams per 100 cubic centimeters of serum is 40 or more, rickets is absent; that when the product is less than 30, rickets is always present, and that when the product is between 30 and 40, rickets may or may not be present. It is recognized that the product thus obtained does not represent a true solubility product of calcium phosphate in the chemical sense and that in all probability other substances in the blood besides calcium and phosphorus are concerned with the process of calcification. Thus, we know that the concentration of sodium chloride and protein can influence the precipitation of calcium phosphate and that in as yet some unexplained way iron is concerned in the process of lime salt deposition. The experiments of Bosanyi (12) on the effects of adrenalin given to rachitic rats demonstrated that rickets might begin to heal even when the calcium-inorganic phosphorus product was as low as 24. Nevertheless, the product obtained by the method of Howland and Kramer is an exceedingly useful working rule and it is this product which will be used in making an analysis of the blood-serum findings in the present group of cases. The discrepancies in the rule when viewed from a chemical aspect should merely warn us that Howland and Kramer's product is not an infallible guide as to the presence or absence of active rickets, especially when the figure is close to the doubtful zone between 30 and 40.

In the series of cases forming the basis of this report no analysis was attempted of such clinical signs of rickets as craniotabes, enlarged costochondral junctions, wrist epiphyses, and others. Although of

[4] The serum calcium was determined by the method of Kramer and Tisdall (*J. Biol. Chem.*, **47**: 475–481, 1921); the inorganic phosphorus by the Briggs' modification of the Bell-Doisy method (*J. Biol. Chem.*, **53**: 13–16, 1922).

unquestioned clinical value, these signs are of secondary importance when compared with the major findings by x-ray and blood chemistry.

ROENTGENOGRAMS AND BLOOD ANALYSES

Between June 12 and December 14, 1924, 260 patients were enrolled. Owing to a variety of circumstances[5] this number was depleted so that at the expiration of the six months' test period 47 patients only were available for study. Roentgenograms of the extremities were obtained in all of these cases; in 43, blood was obtained for chemical analysis. Nine of the patients belonged to the control group, that is, the mothers had taken no cod-liver oil; the rest were infants whose mothers had taken amounts of cod-liver oil varying from 12 to 58 ounces during the six months. Roentgenograms and blood analyses were obtained as closely as possible to the age of six months. For purposes of analysis, when there was any considerable variation from this age, the total amount of cod-liver oil taken by the mother was utilized to give a proportionate figure for the amount taken in six months. The data concerning all of the cases have been tabulated in Table I.

On inspection, individual cases can be found which show wide variations in the amount of rickets present, regardless of the amount of cod-liver oil taken by the mother. Thus, in Case 40 in which the mother had received no cod-liver oil, the infant showed no evidences of rickets by x-ray and had a serum content of 10.0 mgm. of calcium and 6.4 mgm. of inorganic phosphorus per 100 cc. Again in Case 47 in which the mother had received 48 ozs. of cod-liver oil the roentgenograms of the infant's extremities showed advanced rickets (grade 3) and the blood serum calcium and phosphorus determinations were 10.3 and 2.46 mgm. per 100 cc., respectively. This variation is scarcely more than can be expected. It is known that many breast-fed babies do not develop rickets when no attempts at prophylaxis have been made; we can surmise that the inherent susceptibility to rickets (inherited reserve of vitamin D ?) varies greatly and that an amount of vitamin

[5] In a large number of instances the babies were weaned or given accessory feedings during the first few months of life; in other cases, because of change of residence, the patients could not be located; in a few instances the mother gave cod-liver oil directly to the infants.

TABLE I

CASE	AGE	WEIGHT	DATE OF EXAMINATION	C. L. O. TAKEN BY MOTHER IN 6 MONTHS	X-RAY FINDINGS	Ca PER 100 CC. OF SERUM	P PER 100 CC. OF SERUM	Ca × P
	months	*pounds*		*ounces*		*mgm.*	*mgm.*	
1	5	16.2	1/ 8/25	36	1.0	8.4	4.60	38.6
2	7	15.0	1/21/25	33	0.0	10.1	6.10	61.6
3	6	13.1	1/28/25	0	1.0	10.3	3.67	37.8
4	7½	14.7	2/ 4/25	0	2.0	10.0	2.69	26.9
5	7	15.5	2/ 4/25	33	1.0	7.8	3.90	30.4
6	6½	16.0	2/ 4/25	18	3.0	8.7	2.90	25.2
7	6½	14.7	2/ 4/25	26	2.0	Blood for analysis refused		
8	7	21.0	2/ 9/25	45	0.0	11.0	5.85	64.4
9	7	19.2	2/10/25	31	3.0	Blood for analysis refused		
10	7	18.1	2/11/25	0	1.5	8.4	3.54	29.7
11	7	15.8	2/11/25	30	1.0	8.8	3.94	34.7
12	6	15.5	2/18/25	12	2.0	8.0	3.47	27.8
13	6	15.1	2/25/25	28	3.0	9.7	2.90	28.1
14	6½	19.9	2/25/25	0	3.0	10.0	2.40	24.0
15	5	18.2	3/ 2/25	26	2.0	10.4	4.20	43.7
16	7	19.4	3/ 9/25	14	3.0	7.0	3.65	25.5
17	6½	11.0	3/18/25	41	0.5	11.0	4.50	49.5
18	6	20.7	3/18/25	28	1.0	10.5	3.88	40.7
19	6	17.8	3/18/25	0	3.0	9.7	3.46	33.6
20	5	11.2	3/18/25	54	0.0	10.9	5.00	54.5
21	5	12.9	3/20/25	18	2.0	9.5	2.90	27.6
22	9	16.3	3/25/25	45	0.0	10.6	4.00	42.4
23	6	16.0	3/25/25	38	0.0	10.4	5.00	52.0
24	6	17.5	3/25/25	30	1.0	10.5	4.10	43.1
25	6	14.1	3/25/25	40	0.5	10.0	4.30	43.0
26	6	14.9	3/25/25	48	0.5	9.8	4.40	42.0
27	5	12.1	3/25/25	34	2.0	10.0	3.24	32.4
28	6	25.0	3/26/25	28	2.0	10.2	4.00	40.8
29	8½	15.7	4/ 1/25	25	0.0	10.4	5.60	58.2
30	5	14.1	4/ 2/25	58	2.5	10.8	3.10	33.5
31	6	18.7	4/ 8/25	50	0.0	Blood for analysis refused		
32	5½	?	4/ 8/25	20	0.0	10.6	6.40	67.8
33	6	17.3	4/15/25	52	0.0	10.4	6.60	68.6
34	6	15.7	4/15/25	40	2.5	10.0	3.70	37.0
35	6	19.5	4/18/25	28	3.0	10.6	4.10	43.5
36	7	15.5	4/22/25	24	2.0	7.0	5.90	41.3
37	6	18.5	4/22/25	36	0.0	Blood for analysis refused		
38	6	11.6	4/22/25	38	2.0	7.5	4.10	30.7
39	7½	18.0	4/29/25	16	3.0	7.9	4.00	31.6
40	5½	13.0	4/29/25	0	0.0	10.0	6.40	64.0

TABLE I—*Concluded*

CASE	AGE	WEIGHT	DATE OF EXAMINATION	C. L. O. TAKEN BY MOTHER IN 6 MONTHS	X-RAY FINDINGS	Ca PER 100 CC. OF SERUM	P PER 100 CC. OF SERUM	Ca × P
	months	*pounds*		*ounces*		*mgm.*	*mgm.*	
41	5½	10.4	4/29/25	31	1.5	10.0	3.90	39.0
42	6	18.0	5/ 2/25	0	3.0	10.4	2.80	29.1
43	5	?	5/ 6/25	0	2.5	8.0	3.80	30.4
44	6	15.6	5/13/25	20	1.0	10.0	5.50	55.0
45	6	15.9	5/13/25	0	1.0	10.1	4.20	42.4
46	6	15.0	5/20/25	56	0.5	10.6	5.10	54.1
47	5½	11.9	5/27/25	48	3.0	10.3	2.46	25.3

D sufficient for prophylaxis in one case may be quite insufficient in another. Furthermore, it is possible that in spite of efforts to gauge the amount of oil taken by each mother, parts of it may bave been used for other purposes, and the recorded estimate be incorrect. It is therefore evident that little can be expected from a study of individual cases and that an analysis must be made on the average findings in the various groups. By this means the influence of extraneous factors on the development of rickets may be equalized. The possible significance of several of these factors will be discussed presently.

In Chart I the cases have been arranged in groups according to the amounts of cod-liver oil taken by the mothers, and these amounts plotted as abscissae on a curve. The ordinates for each group represent the average calcium-phosphorus product of all the cases in each group as determined by analysis of the blood serum. It can be seen that this average product, except for a slight fluctuation in the group whose mothers took 30 to 40 ounces of oil, becomes greater as the amount of cod-liver oil taken by the mothers increases. This means that the average degree of rickets in the infants as estimated by analysis of their blood serum for calcium and inorganic phosphorus varied inversely with the amount of cod-liver oil taken by the mothers, and would suggest that this cod-liver oil had furnished the infants through their mothers with a measurable amount of antirachitic substance.

From Chart II, based on a similar analysis of the roentgenographic findings, the same conclusion can be drawn. In general the greater the quantity of cod-liver oil taken by the mothers the less the de-

gree of rickets in the infants as estimated by x-ray pictures of the extremities.

In contrasting the results obtained by blood analyses with those by skeleton roentgenograms it is well to remember that the former give a cross-section picture, that is, an estimate of the activity of the disease at the time of drawing the sample of blood, whereas the latter afford a

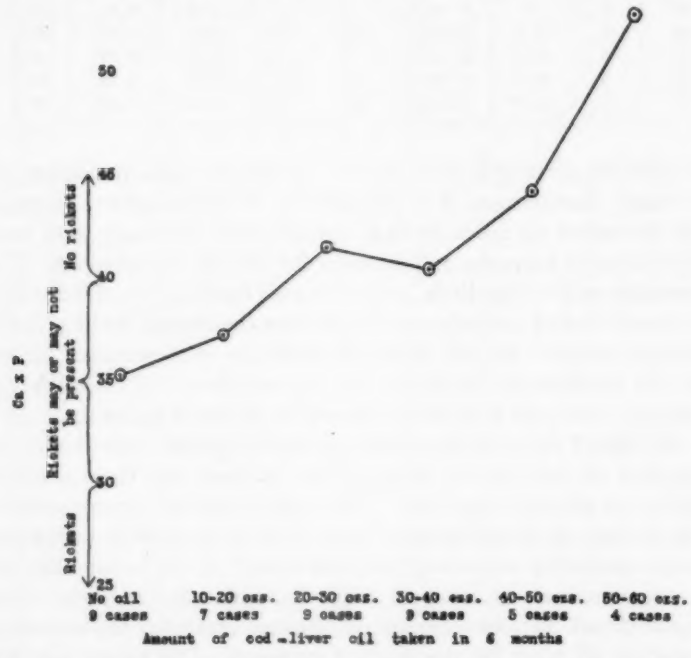

CHART I. ANALYSIS OF BLOOD FINDINGS IN 43 PATIENTS WHOSE MOTHERS TOOK VARYING AMOUNTS OF COD-LIVER OIL IN THE 6 MONTHS' TEST PERIOD

record of past events, so that, even though the disease be healing and the serum findings normal at the time of examination, the story of the past may be read in the x-ray picture. For the purposes of a study of this kind it is evident that the kind of information obtained by roentgenograms is the more desirable; the findings by serum analysis have

the advantage of furnishing an easily mensurable figure. In the present survey both methods of study gave concordant results.

In a statistical study of this kind it is evident that certain uncontrollable factors will have played a part in protecting or predisposing some of the infants to rickets. No attempt was made to regulate the diets of the mothers in regard to other foods containing vitamin D and no attempt was made to control the amount of exposure to sunlight of either mother or infant. It was hoped merely that the influence of such factors, so important in the individual case, would be minimized by the method of studying averages.

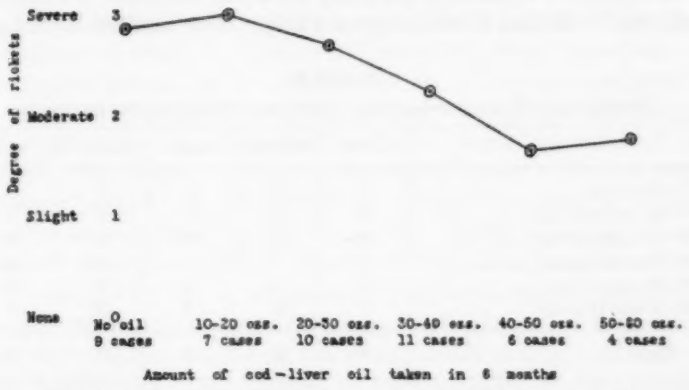

CHART II. ANALYSIS OF ROENTGENOGRAMS OF EXTREMITIES OF 47 PATIENTS WHOSE MOTHERS TOOK VARYING AMOUNTS OF COD-LIVER OIL IN THE 6 MONTHS' TEST PERIOD

It would seem that any significant effect from the normal seasonal fluctuation of the disease could be eliminated. Hess and Lundagen (13) in New York, from their studies of the average blood findings in a large group of infants, showed that the level of inorganic phosphorus in the blood fell during January and February, reached a minimum in March, rose slightly in April, and significantly in May, to return to normal in June and July. The patients included in this report were all examined between January 21 and May 27th, a time which included the period of greatest activity of rickets in this section of the country, and during which the effects of any prophylactic regime should be

most evident. Moreover, as can be seen from Table II, in each group from which average figures were obtained, the individual cases were widely distributed through the different months of study. In May, the only month included in the study in which a normal seasonal decrease in the disease might have been great enough to lead to a significant error if interpreted as due to therapy, only six patients were examined. Four of these were infants whose mothers took less than 20 ounces of oil and therefore any spontaneous healing of the disease due to seasonal factors should have affected the control cases more than the treated ones.

The patients included in the study were next examined to ascertain whether variations in rate of growth might have resulted in changes

TABLE II

Distribution of 47 cases forming basis of report according to month of examination

	JANUARY	FEBRUARY	MARCH	APRIL	MAY
Control group	1	3	1	1	3
10–20 ounces group	0	2	2	2	1
20–30 ounces group	0	3	4	3	0
30–40 ounces group	2	2	3	4	0
40–50 ounces group	0	1	3	1	1
50–60 ounces group	0	0	1	2	1
Total	3	11	14	13	6

wrongly interpreted as due to therapy. It is known that rickets is a disease, the symptomatology of which depends upon skeletal growth, and that starvation effectively stops the progress of the disease. For this reason we might expect on the average to find less evidence of rickets in infants who from disease or other causes had been retarded in growth than in those of normal or greater than normal size. Regardless of the general importance of this growth factor, it would seem to have had little or no effect in the group of infants under discussion, at least if body-weight may be taken as an index of skeletal growth.[6] In seven cases in which the infants were 20 per cent or more below

[6] It is unfortunate that measurements of body-length were not made, as these would have furnished a more satisfactory index of growth rate.

the normal weight for their ages the average roentgenographic figure indicative of the degree of rickets was 1.43 as compared with a figure of 1.33 in a group of nine infants more than 10 per cent above normal weight. The same group of 7 undernourished children had an average calcium-phosphorus product of 38.2, while the average product of 6 of the overnourished infants in whom blood analyses had been made was 42.0. In other words, the degree of rickets was slightly greater in the most poorly nourished infants of the group than in the well-nourished.

SUMMARY

In reviewing the results of this survey it will be seen that it has been necessary to adopt a method of analysis which places importance on degrees of intensity of the rachitic process. It has not been possible to show that the administration of cod-liver oil to the mother in a dose of practical size is a satisfactory method of preventing rickets in the infant. The remedy is evidently far less efficacious than when given directly to the infant. Nevertheless, the figures obtained do indicate that, *when cod-liver oil is given to a mother, a certain amount of the anti-rachitic vitamin does pass into her milk* and that, even though in individual cases the amount so transmitted to the infant may be insufficient to prevent manifestations of the disease, nevertheless, when the average degree of rickets existing in a group of such infants is considered, the transfer has been sufficient to raise considerably the calcium-inorganic phosphorus product of the blood serum and to lessen the roentgenographic evidences of disease.

The author wishes to express his thanks to the many friends who coöperated to make this survey possible,—to Mr. E. M. Johnson of Mead, Johnson and Company for generously providing the cod-liver oil, to Miss Frances Etchberger of the Babies Milk Fund Association, to Dr. Mary Willis of the Bureau of Child Hygiene of the City Department of Health, and to the nurses of these organizations for providing adequate follow-up work in a large group of patients, to Dr. P. G. Shipley for checking the antirachitic potency of the cod-liver oil, and to Mr. I. Gittleman and Dr. J. B. Pincus for the serum calcium and phosphorus analyses.

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BOOK REVIEWS

The Diseases of Infancy and Childhood. By L. EMMETT HOLT, M.D., Sc.D., M.D., and JOHN HOWLAND, A.M., M.D. Ninth Edition. 1018 pp. $10.00. (*D. Appleton and Co., New York and London, 1926.*)

The death of Dr. Howland during the last summer caused me to pay particular attention to the new edition of this textbook as representing a last message from this illustrious physician and teacher. Dr. Park of Yale tells us in the preface that since the preceding edition both Dr. Holt and Dr. Howland have died, and that Dr. Howland had completed the revision of the present edition before his death. He adds that although almost every page had been changed or rewritten by Dr. Howland, the foundation of the book—its plan, arrangement, proportions and general character—remains as the work of Dr. Holt. Thus, although the book does not suggest Dr. Howland as seen by his students in his clinical demonstrations so well as certain of his other articles (as his chapter on Measles in Oxford Medicine), one recognizes that it expresses the fundamental facts and experience absorbed by Dr. Howland during his life.

With so many revisions, the book has tended to become an accumulation of facts and has lost much of the personality of the authors as expressed in its literary style. This is not necessarily a fault; the change renders it better adapted for reference; it is not so attractive for easy reading during spare moments.

On comparing this and the preceding edition, I first noticed that instead of 1127 pages, there are now only 1018; I soon discovered that this diminution is apparent rather than real, for the printed part of each page of the new edition is ½ inch longer and ½ inch wider. There has been a deletion of practically every needless phrase and sentence; thus the conciseness of the text is more marked. The following chapters have been changed in general plan of presentation and fundamental statements—Pneumonia, Congenital Anomalies of the Heart, Acute Nephritis, Cerebral Tumors. The newer methods of treatment in Scarlet Fever and Diabetes are discussed. New chapters have been added on Enlarged Thymus and Erythredema. Of the minor changes, I would mention (1) the change of the chapter title from Hereditary Syphilis to Congenital Syphilis, (2) the omission of the description of the method of analysis of breast milk, (3) the omission of the section dealing with top-milk mixtures and (4) the recommendation of the use of ether instead of chloroform in the treatment of convulsions.

The ninth edition is a distinct improvement over the previous ones. The book is still to be regarded as the standard authority on Pediatrics and will doubtless long remain a monument to the scientific and clinical ability of its authors.

H. L. H.

Hydrogen Ion Concentration. By DR. LEONOR MICHAELIS, translated into English by Dr. William A. Perlzweig. 299 pp. $5.00. (*The Williams & Wilkins Co., Baltimore, 1926.*)

In the present medical era we are frequently perturbed both by the clinical writings of physicists, chemists, statisticians, and others—men of sound laboratory training but with lack of experience in and therefore of appreciation of the real problems of clinical medicine—and also by the efforts of the clinician of limited training in the pure sciences to plunge boldly into the domains of physics, chemistry, mathematics and whatnot. All are agreed that the modern advances in these branches may offer solution to some of our most baffling clinical problems, and yet the individual with the breadth of experience in both fields necessary to furnish the proper criteria in separating the essential from the non-essential is rarely encountered. It is with these thoughts in mind that the reviewer has been particularly glad to see an English translation of Dr. L. Michaelis' "Wasserstoffionenkonzentration." Dr. Michaelis' early training as a physician has given him interests and a point of view reflected in his writings through many years devoted to physical chemistry and allied biological problems. His book on hydrogen ion concentration, therefore, while bearing on the scientific side the stamp of authority of one of the creators of the branch of chemistry known as physical chemistry, at the same time abounds in examples illustrating the application to medicine of the principles which are discussed, and is fertile with suggestions concerning medical obscurities needing further physico-chemical investigation. As illustrative of one of these examples the discussion of the solubility of calcium in blood serum in the chapter devoted to "The relation of hydrogen ion concentration to the solubility of difficultly soluble salts" may be cited. The concluding lines of this discussion are worthy of being quoted: "It may be seen . . . . how much more important is an exact knowledge of natural constants than are hypotheses for the clear conception of physiological facts. It is surprising that such physiologically important constants have not yet been determined. The theories of formation of calculi and of the deposition of lime in the blood vessels are truly phantastic, as long as it is not established whether or not the calcium of the blood is at all in a supersaturated state."

The translation contains, in addition to the material of the German edition of 1921, a number of addenda by the author indicating the more important recent advances in the subject. The subjects specially noted in these addenda are, the contributions to the activity theory of ionization by G. N. Lewis, Bjerrum, and Debye,—the modification of the theory concerning the dissociation of the ampholytes by Bjerrum,—the theory of oxidation-reduction potentials, with especial reference to providing a basis for understanding the quinhydrone electrode.

The translation has been put in a very readable English and will be welcomed by those who have difficulty with the intricacies of scientific German.

A. A. W.

The Normal Child and How to Keep It Normal in Mind and Morals. By B. SACHS, M.D., 111 pp. $1.50. (*Paul B. Hoeber, Inc., New York, 1926.*)

This book of 105 pages contains 50 printed pages devoted to The Normal Child, and 38 printed pages devoted to a chapter entitled *The Evils of Psychoanalysis.* This final chapter seems a fitting culmination to the sly thrusts at the psychoanalytic school abounding in the previous chapters, so that one gets a feeling, after closing the little book, that he has read a brief on the subject of psychoanalysis. The Normal Child is discovered by the simple process of eliminating him from "the nervous child, the defective child, the delinquent, the truant child, the slightly erring boy and girl and the criminal youth." Having divided childhood neatly into normal and abnormal, the author goes on to reconstruct "infancy, the pre-school period, the school age, and puberty and adolescence." The problems of infancy are covered by attention to regular habits of feeding, sleeping, and keeping the infant free from excitement. In discussing the pre-school period, the writer urges a wholesome balance of play and exercise and amusement; the avoidance of stories or pictures causing fear or terror. "Every child should be made to feel the weight of authority." Dr. Sachs looks to school and teachers "for the development of character and the proper stimulation of the latent energies." The brightest jewel in the crown of the modern school is that it does not overwork its students. Puberty and adolescence is a time when consideration of sex matters can be decently undertaken. The father should give the son sex instruction, and the mother, the daughter. "Physician and parents should make every effort to curb any attempt at self-abuse in boys as well as girls. It should not be countenanced and certainly not condoned." As for ambitions, strivings and plans, "When the time comes for son or daughter to determine upon a life career, let the parent or teacher guide the individual unselfishly and with calm judgment." Here again we assume that all is easily and quickly adjusted.

In this discussion of the so-called Normal Child, the writer seems to ignore any points of view with regard to the behavior of children that have developed in the last thirty years. Childhood behavior is still a matter of "mind and morals." "The salacious doctrines of the Austrian school" are, after all, only one of many developments in the psychology of childhood. One misses any critical study of the factors influencing the growth of personality—personal conflicts, interparental disagreements, false social standards, and many other common causes of maladjustment. Surely there is a happy mean between the complexities of psychoanalytic hypotheses and the cut-and-dried-rules-and-regulations attitude toward the child that was the accepted educational philosophy of a generation ago.

E. L. R.

Politzer's Textbook of Diseases of the Ear. 6th ed. Revised and largely rewritten by Milton J. Ballin. 776 pp. $10.00. (*Lea & Febiger, Philadelphia, 1926.*)

The sixth edition of Politzer's textbook, "Diseases of the Ear," has for the third time been revised by Ballin. As the editor tells us in the preface, the book

has been very much condensed but still gives a very comprehensive survey of the field of otology.

The illustrations are numerous and helpful but many of them could be improved upon, especially the drawings of the internal ear and of the various operative procedures on the mastoid.

The many references to original articles render the work of special value to the active otologist; unfortunately, the average undergraduate medical student may still find it too long.

To recapitulate, this book gives a very good survey of the field of otology in a style that is simple and direct. The few slight faults can be explained by the fact that revisions, made after the original author is deceased, tend to represent neither the entire thought of the author nor that of the editor: many topics must be retained for sentimental reasons that would probably be discarded as having served their purpose were the editor writing a book entirely his own.

J. W. B.

Manson's Tropical Diseases—A Manual of the Diseases of Warm Climates. Edited by Philip H. Manson-Bahr. 8th Edition, Revised. 895 pp. $11.00. (*Wm. Wood, New York, 1925.*)

First published in 1898, this classic of modern medical literature has now reached its 8th edition. The 6th edition, published in 1917, was the last one for which Manson was solely responsible. In 1921 he died at 78 and the 7th and 8th editions have been prepared by his son-in-law, P. H. Manson-Bahr, who had also assisted in the preparation of several former editions.

The year 1898 marked the official entrance of America into the field of Tropical Medicine, and Manson's book therefore appeared at the psychological moment for American physicians in the Tropics. His teachings have had a powerful and wholesome effect on American practice. Just as the Pilgrim Fathers went out to conquer the new continent with the Bible in one hand and a musket in the other, so in the eager years following 1898 the American medical man went into the Tropics of the East and West Indies and Panama with "Manson" in one hand and a microscope in the other, and conquered, or at least recognized, many strange diseases. Every few years new editions appeared which kept the book up to date scientifically and in America it has held its early popularity, at least until recently. The reason for the success of "Manson" was its balance between clinical and laboratory medicine. Manson, from long residence in the Tropics, had a first hand knowledge of patients and conditions, and he also took a leading part in the scientific work which has thrown so much light on various tropical diseases. His preparation and poise were Darwinian. The subject, of course, underwent a tremendous development from increased knowledge of the specialties of protozoology, bacteriology, helminthology, entomology and chemistry. Medical literature became flooded with writings from the pens of workers who knew the Tropics only from the deck of a boat, from an intensive expedition, or from

maps, journals, specimens and parasites—far from the heat and labor of the day. The handling of the individual patient or situation was more or less lost sight of. But Manson always kept the balance between the single-track scientist and the clinical scoffers. The various editions were not infallible, as the 5th gave undue prominence to some mistaken views on pellagra—but the book remained the personal reaction of a master mind.

It is evidently desirable to preserve such a work as long as possible, but it must be a serious problem for an editor of an "Osler" or a "Manson" to decide how to proceed. On the one hand, there is a vast increase in the specialties which make up the sum total of modern medicine and it is increasingly difficult for one man to cover the whole field. On the other hand, the value of such works as Osler and Manson to the average individual lay in the personal selection and attitude of the author. Even in Manson's time, however, the book grew larger and larger and became over-loaded with systematic zoological classifications of parasites which were forbidding to the average doctor. Some of us began to wonder what would or could become of the original purpose of a "manual of handy size and yet giving adequate information."

In the meantime two other single outstanding books have appeared on the field, Castellani and Chalmers in 1910, and Stitt in 1914. The former authors disregarded size, gave full play to systematic considerations and produced an encyclopaedic work of dictionary size. Stitt has consistently adhered to the idea of giving students and practitioners a "concise and readily accessible presentation." Instead of one book, therefore, there are now at least three of this kind that the English speaking doctor can consult. Each has its merits and limitations, with Manson occupying a middle position as regards size. Mere weight is, of course, a very crude way of evaluating medical literature, but a manual, strictly speaking, should be handy. The present edition weighs 3¼ pounds as contrasted with Stitt's 1½ pounds and Castellani and Chambers' 6¼.

In the present edition there is a welcome move to keep the volume from becoming more unwieldly by a reduction of seventy pages. Manson's introduction, which had been omitted since the 6th edition, is also restored. This move is in the right direction and could be carried further. The book at present has 628 pages of text and 236 of appendix. It contains many of Manson's original paragraphs and sentences with outcroppings of additions of various literary strata. Possibly, in future editions the reading physician would prefer to see the spirit preserved at the expense of the form and the whole work welded into a concise, homogeneous and integrated entity. For example, a change might be made in the time-honored classification of Section II on "General Diseases of Undetermined Nature" which includes Beriberi, Pellagra and Scurvy, none of which is, strictly speaking, of undetermined nature.

An excellent feature of the present edition is the addition of many new illustrations of lesions and parasites, some of which are beautifully colored and distinctive. A few older illustrations are retained which are principally of historical

value. There are also six maps which show the geographical distribution of trypanosomiasis, sleeping sickness, Leishmaniasis, Schistosomiasis, yellow fever and filariasis. In spite of the sketchy or "skeleton" nature of these maps, they are a valuable addition.

American readers will be interested in the fuller acceptance of the lepto-spiral etiology of yellow fever and the grouping of yellow fever, infectious jaundice and seven-day fever under the one head of Lepto-spirosis. The malarial treatment of paresis receives notice and the light shed on malaria by these studies will doubtless receive more attention in a later edition. The recent important culture of pathogenic amoebae is also mentioned and will also require more space later. In general, therapeutics and prophylaxis, which are the goals of scientific medicine, are well covered.

In spite of all the possible pros and cons, this edition holds its own as a fine presentation of Tropical Medicine of today. It is of great value for the tropical practitioner who wishes "adequate information" but who cannot pack around a library on the specialties.

H. J. N.

THE INACTIVATION OF THE CHICKEN-TUMOR VIRUS BY MEANS OF CARMINE[1]

MARGARET R. LEWIS AND HOWARD B. ANDERVONT

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In 1926 Lewis and Andervont found that the washed white blood-cells from a tumor chicken injected into the breast of a normal one brought about the formation of a tumor. At that time an effort was made to follow the subsequent behavior of these leucocytes in order to determine whether the injected cells became transformed into malignant cells, or whether they carried a virus which caused the tumor. For this purpose 5 cc. of blood, drawn from a tumor chicken, were mixed with a suspension of carmine and then centrifuged. The white blood-cells took up the particles of carmine, and after they had been washed with Locke solution most of them contained one or more bright red particles by means of which it was hoped to identify them after injection (Eliot, 1926). Unfortunately, instead of furnishing a means of identifying the leucocytes, the carmine prevented the formation of the tumor. Previous studies (Lewis and Lewis, 1926) of the behavior of the macrophages made it seem unlikely that the injection of the carmine particles had interfered with the usual activities of the cells. It seemed more probable that the phenomenon might be one of adsorption, that is, that when the carmine particles were taken into the cells they came in contact with the tumor-producing agent and adsorbed it. This led to the present investigation of the effect of carmine on the chicken-tumor virus *in vitro*.

The strain of tumor used in these investigations was obtained by injecting the breast of a chicken with a small amount of the dried tumor powder, originated by Murphy (Rous, 1911). After a tumor had been obtained in this way, it was continued from one chicken to another by injecting a tumor extract or by transplanting a piece of tumor tissue.

[1] Received for publication February 26, 1927.

The virus present in each tumor necessarily varies with the individual chicken, and, since there is no method of obtaining the pure virus, it was difficult to prepare a standard amount of the virus for experimental purposes. As a large part of the virus is lost in making the filtrate, it was decided to use a simple extract of the tumor made as follows: 3 grams of tumor, together with fragments of glass, were ground in a sterile mortar until the tissue was reduced to a smooth paste; 30 cc. of sterile Locke solution were added; the solution was well mixed and centrifuged for five minutes, after which the supernatant fluid, the extract, was taken off into sterile tubes. The extract prepared in this manner was, in general, cell-free, but no effort was made to be certain that it was absolutely so.

Dry carmine was ground to a fine powder in a hand mortar, weighed, placed in sterile tubes, stoppered and Arnoldized. Carmine obtained from different sources, Grübler, Coleman, Merck and Muth, differed markedly in the size and shape of the carmine particles and in its solubility in the tumor extract. For the sake of uniformity it was decided to limit the present study to the effect of Merck's carmine, although it was found that the other brands also inactivated the tumor virus.

Two cubic centimeters of tumor extract were added to the carmine and mixed by drawing it up and down through a capillary pipette or by shaking it until it became an even suspension. This was centrifuged for five minutes and then the supernatant fluid was removed to another sterile tube. The deposit was washed by mixing it with 5 cc. of Locke solution after which both this and the supernatant fluid were again centrifuged. The Locke solution was then poured off the deposit and 2 cc. of fresh Locke solution were added, mixed with the deposit, and the suspension injected into the breast of a chicken. Since it has become the accepted technic to add a trace of kaolin to the filtrate, when injecting it in order to produce a tumor, the supernatant fluid had a trace of kaolin added to it before being injected.

In some experiments, for the sake of economy, the same chicken received both the supernatant fluid and the deposit, that is, the supernatant fluid was injected into one side of the breast and the washed deposit into the other. Some of the chickens which failed to form a tumor within six weeks were used as controls for later experiments.

Carmine was tried in 50, 33, 27, 25, 21, 15, 10, 5, 2.5, and 1.25 per cent suspensions. All of these, except the 1.25 per cent, rendered the virus inactive, that is, the virus not only was removed from the supernatant fluid and carried down with the carmine particles when centrifuged, but it was so completely linked with the carmine that even the deposit, when injected into a chicken, failed to produce a tumor.

Tumors from 15 chickens were used to prepare the extracts for the study of the effect of carmine on the virus. After the preparation of the tumor extract with the desired percentage of carmine had been completed and the supernatant fluid and the deposit had been injected, 2 cc. of the extract were injected into another chicken to determine the activity of the virus in the extract used. The period of time necessary to prepare the carmine-inactivated extracts did not perceptibly attenuate the virus, since the control chicken always developed a tumor in about ten days or two weeks. To test further the period of potency of the virus, a tube of extract was left at room temperature for thirty hours before it was injected. The chicken, however, developed a tumor as usual, showing that the virus had not suffered any injury during its prolonged stay outside the chicken. The extracts used were extremely virulent and in the control animals always produced a tumor which metastisized and killed the chicken after a month or six weeks.

In order to ascertain whether the carmine might simply have carried down the virus and not actually combined with it in the suspension, extracts of five tumors were prepared, each containing a desired amount of carmine. After being mixed, but not centrifuged, they were injected as suspensions. These contained, respectively 50, 25, 12.5, 10, 5, 2.5, and 1.25 per cent of carmine. In every instance the chicken injected with the extract alone produced a tumor, but only those which received the mixture of tumor extract and 1.25 per cent carmine did so. It is probable that less carmine would have consistently inactivated the virus had it been possible to prepare it as an even suspension of minute particles.

In an effort to compare the effect of other well known adsorbents on the tumor-producing agent with that of carmine, some of the same extracts of tumor, as were used with carmine, were mixed with Fuller's earth, with powdered charcoal, with kaolin, with powdered Berkefeld

filter and with India ink in 50, 25, and 10 per cent suspensions, without in any instance inhibiting the ability of the extract to produce a tumor, even when as much as 50 per cent of the adsorbent material was added. In two instances, once with 50 per cent Fuller's earth and once with 25 per cent animal charcoal, the virus was removed from the solution so that the supernatant fluid did not bring about the formation of a tumor, although the deposit did. The same percentage of charcoal and even a higher percentage of this material, when again tried, failed to absorb the virus.

It then became important to ascertain whether the carmine had some effect upon the virus other than that of adsorption. Carmine powder is a complex material, as it is made from an extract of the cochineal bug (coccus cacti) precipitated by means of tin or aluminium, lime and certain metal acids. The process is secret and differs according to the manufacturer preparing it. However, it was possible to test a number of different solutions of the carmine itself, such as, Kiyona's lithium carbonate solution (1914), which contains 5 per cent carmine, borax carmine which has the same amount of carmine, and sodium bicarbonate carmine which, while it contains less carmine, is not so irritating as the other two, since it can be prepared with only 0.5 per cent of sodium bicarbonate. While each of these solutions contained many times more carmine than was dissolved in the tumor extract, and the Kiyona's carmine had as much carmine in solution as there was carmine powder present in some of the suspensions used, none of them, when added to the tumor extract, inhibited it from forming a tumor; in fact, all of them aggravated the growth of the tumor and brought about an earlier appearance as well as a more extensive and more rapid growth. Since this may have been due to the presence of the alkali necessary to dissolve the carmine, an effort was made to obtain a neutral solution by dissolving as much as possible of 1 gram of carmine in a liter of distilled water. This solution was then evaporated down to 50 cc., and 0.45 gram sodium chloride was added. It did inactivate the tumor virus in one instance, where 75 per cent of it was added to the extract, but failed to do so in three other instances, two where 66 per cent and one where 50 per cent was added. It was found that this solution frequently precipitated out upon standing, and so it is probable that,

in the one instance in which it prevented the formation of a tumor, it had been precipitated when added to the tumor extract. A saturated extract of the cochineal bug also failed to prevent the virus from causing the growth of a tumor, except in one instance, in which the extract was made by substituting cochineal solution for Locke solution, making 100 per cent cochineal. One per cent carminic acid in 0.9 per cent sodium chloride, used in place of Locke solution in preparing the tumor extract, or added to the tumor extract in equal parts, failed to prevent the appearance of a tumor. Since these various solutions of carmine failed to inhibit the growth of the tumor, it seems reasonable to conclude that the inactivation of the tumor virus is due not to the action of the dye principle in solution, but to the adsorption of the virus by the particle.

An effort was made to release the adsorbed virus by dissolving the carmine deposit by means of saturated lithium carbonate. So far these experiments have failed.

Experiments which are now in progress indicate that the aluminium is the important substance in the particle, since a suspension of aluminium hydroxide and tumor extract failed to bring about the growth of a tumor while the tumor extract alone produced a large tumor within six days.

SUMMARY

From these experiments it may be concluded (1) that the active principle in extracts of chicken tumors is inactivated by carmine, and (2) that this loss of ability to produce a tumor is due to the adsorption of the virus by the carmine particles.

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THE PASSIVE TRANSFER OF HYPERSENSITIVITY (LOCAL AND CONTRALATERAL PASSIVE TRANSFER EXPERIMENTS)[1]

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In 1921 Prausnitz and Küstner (1) first demonstrated that a hypersensitive state could be transferred to a skin area of a normal individual by an intradermal injection of the serum of a sensitive patient. With the blood serum of a patient sensitive to fish the authors were able to sensitize passively the skin of a normal individual. They were unable to obtain the same results with blood serum from patients sensitive to horse serum and pollens. De Besche (2) succeeded in sensitizing the skin of normal individuals passively to horse serum and to pollen by the injection of blood serum from patients sensitive to these substances. Coca (3) and his associates have further advanced the study of passive transfer of hypersensitiveness, and have been able to demonstrate by this procedure atopic reagins (discovered by Prausnitz and Küstner) in the blood of all patients subject to hay-fever and asthma, who reacted to the intracutaneous injection of atopens. They found that skin sensitiveness conferred passively may last four weeks. Passive transfer with the serum of individuals suffering from drug idiosyncrasies could not be accomplished by these investigators. Longcope, O'Brien and Perlzweig (4) have shown that two proteins, precipitating out of colloidal solutions at different isoelectric points, can be isolated from crude extracts of horse-dander; that these proteins act as antigens in guinea-pigs, and that by the Dale technique they can be differentiated by their anaphylactic reactions. O'Brien (5) has further shown that the skin of a normal individual could be readily sensitized by the injection of blood serum from a horse asthmatic and that these sensitized areas of the skin would react specifically to the two protein fractions of horse-dander extract.

[1] Received for publicat'on March 1, 1927.

The following experiments were undertaken in order to determine (1) the variation in the sensitizing power of serum from "timothy" hay-fever patients; (2) the effect of heat on sensitizing serum; (3) the duration of local passive sensitiveness; (4) the effect of repeated injections of a single extract into one sensitized area; (5) the effect of combining sensitizing serum and specific extract before injection; (6) the contralateral passive transfer of hypersensitiveness with the serum of patients sensitive to timothy pollen, ragweed pollen, and rabbit hair; (7) the possibility of sensitizing passively guinea-pigs with serum from patients sensitive to rabbit-dander and horse-dander.

Technique. The blood was obtained from the median vein and after clotting was centrifuged under sterile precautions. The serum which was obtained was not passed through a Berkefeld filter. It was kept in the ice-box without the addition of preservatives. Numerous cultures were made at intervals varying from one day to four weeks after bleeding to determine the sterility. The sensitizing injections consisted of 0.1 cc. of serum and the provoking injection of pollen extract was not more than 0.05 cc. The injections were made on the anterior surface of the forearm. The position of the site in reference to the elbow was noted and where comparisons were made, corresponding sites on the two arms were used. The reactions were read after 15 minutes. Two measurements were made both of the wheal and of the erythema, one parallel to the length of the arm, the other at right angles. In the tabulations the average of the two readings was used. Pseudopodia were not included in the measurements.

Variations in the sensitizing powers of the serum of hay-fever patients sensitive to timothy extracts. The sensitizing power of the serum did not parallel the clinical severity of the disease. With 0.1 cc. of the serum from one patient (C)[2] who usually experienced mild symptoms, the skin of a normal individual was sensitized by 1 in 512 dilution. This patient (C), incidentally, had not received treatment for hay-fever in the preceding year. When 0.05 cc. of 1–100 dilution of timothy atopen (Nitrogen content = 46 mgm. per 100 cc.) was injected

[2] The individual (C) showed very marked skin reactions to an intradermal injection of timothy extract. An injection of 0.025 cc. of timothy diluted 1:100,000 with physiological saline (0.00000012 mgm. of N) produced a wheal 1 cm. in diameter surrounded by redness 4.5 cm. in diameter, and with a dilution of 1:1,000,000 a wheal 0.8 cm. in diameter and redness 3.5 cm. in diameter developed. Controls gave no reaction. Precipitin tests (ring method) were faintly positive with this serum. In the other sera of this experiment the results were negative.

into the skin areas of the forearms of normal individuals sensitized with serum from patient (C), large reactions characterized by wheals and redness appeared within 15 minutes; the measurements are tabulated in Table I. The serum of six other hay-fever patients giving a history of severe hay-fever showed much less sensitizing power. These patients in the preceding year had had treatment for their symptoms. The serum of patient (R) sensitized slightly in dilution of 1 in 512 and strongly in dilution of 1 in 128, while the serum of patients R-1, B-a, B-u, and K-a failed to sensitize in dilutions greater than 1 in 4. On the other hand, the serum of the patient

TABLE I

Variations in the sensitizing power of sera taken from patients sensitive to timothy pollen. Reactions to the intradermal injection of 0.05 cc. timothy extract (dilution 1:100) in sensitized sites, time intervals remaining constant (see Fig. 1)

DONOR SERUM	NORMAL RECIPIENT	DILUTIONS OF SENSITIZING SERA									
		1:512		1:128		1:33		1:4		Undiluted serum	
		Wheal	Redness	Wheal	Redness	Wheal	Redness	Wheal	Redness	Wheal	Redness
		cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
C	L	1.0	5.0	1.3	5.3	2.0	5.0	—	—	—	—
	G	1.0	5.25	1.3	5.0	—	—	—	—	—	—
M	Ga	—	—	0.8	1.0	—	—	1.5	6.7	2.0	7.7
R	H	0.9	1.1	1.5	2.5	—	—	—	—	1.9	4.7
Rl	Y	—	—	—	—	—	—	1.0	3.0	1.4	4.0
Ba	A	—	—	—	—	—	—	1.0	2.5	1.2	2.5
Bv	D	—	—	—	—	—	—	1.4	3.0	2.2	4.8
Ka	N	—	—	—	—	—	—	1.5	3.0	1.3	4.0

(C) diluted 1 in 32, sensitized more highly than any of the other sera diluted 1 in 4. This same serum (C) diluted 1 in 32, sensitized more highly than three of the other sera undiluted; equally well as two of the other sera undiluted, and somewhat less than the sixth serum undiluted (Table I, Fig. 1).

We realize that there is considerable variation in receptiveness of the skin of normal individuals to passive sensitization. Nevertheless, we feel that these figures give evidence that the severity of the clinical symptoms does not parallel the amount of sensitizing body present in the serum. Further evidence of this is to be found in the failure of

the serum from six cases (after they had received biweekly prophylactic injections for six weeks) to show a reduction in sensitizing power. This experiment was carried out as follows: A small amount (0.1 cc.) of the serum of the patients was injected into several sites in the skin of the forearms of six normal individuals. After three days 0.05 cc. of two dilutions (1:100 and 1:1000) of timothy extract was injected into two sensitized areas. These reactions were measured. Upon the completion of the treatment of the patients, blood was again drawn and serum injected into the skin of the same normal individuals.

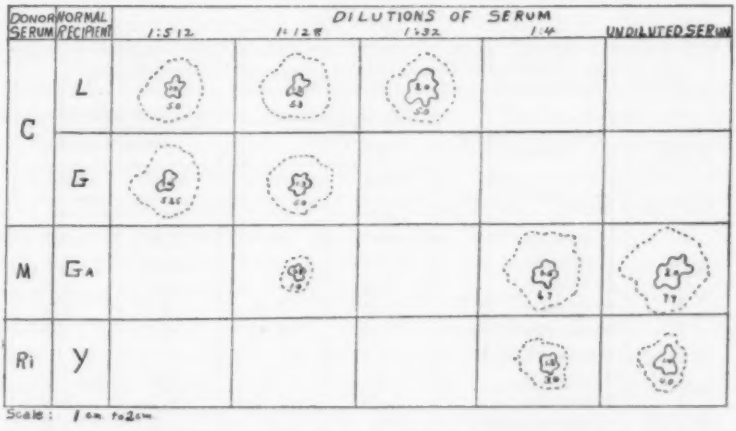

FIG. 1. SHOWING THE VARIATIONS OF SENSITIZING POWER OF SERUM TAKEN FROM PATIENTS SENSITIVE TO TIMOTHY

Similar amounts of diluted timothy extract were injected and the measurements of the reactions following this injection were the same as those previously obtained before treatment. In one case the reaction was considerably increased. Our observations corroborate those of Levine and Coca (6) and we would judge that the sensitizing power of the sera of our six patients was neither influenced by the treatment of the preceding year nor by the six weeks of treatment just preceding the experiment. However, as a result of treatment, clinically, these six patients had what might be considered a season free from symptoms. Further studies must be made before one can

determine why the sensitizing power of the serum fails to change following a course of treatment. Coca states that because the degree of reaction, produced by the above mentioned passive transfer experiment, is unchanged in the same normal individual after a course of treatment, "desensitization" of our patients to foreign protein is not accomplished by our method of treatment. The few cases which he has cited are not sufficient to warrant such a conclusion.

The effect of heat on sensitizing serum. We were able to confirm the statement of Coca and Grove that heat for one-half hour at 56°C.

TABLE II

The effect of heat on sera taken from hypersensitive patients. Comparative reactions following injection of provocative doses of timothy extract into the sensitized sites

SERUM SPECIMEN NUMBER	DURATION OF HEATING (56°C.)	SERUM REACTION DUE TO HEATING		REACTION FOLLOWING INJECTION OF TIMOTHY EXTRACT IN SENSITIZED SITES	
		Wheal	Redness	Wheal	Redness
		cm.	cm.	cm.	cm.
1	5 minutes	0	0	1.0	3.0
2	10 minutes	0	0	0.8	2.5
3	20 minutes	1.0	2.5	0	4.0
4	30 minutes	1.3	3.25	0	4.0
5	60 minutes	1.3	3.25	0	2.5
	Heated 38°–40°C.				
6	30 minutes	0	0	1.0	3.0
7	60 minutes	0.8	1.5	0.7	2.2
8	Unheated control	0	0	1.5	5.0

Timothy extract nitrogen content = 46 mgm. per 100 cc. (Coca's solution extract).

reduces the sensitizing power of the serum. In two experiments the serum from timothy-sensitive subjects was heated for two hours at 56°C., after which small fractions were injected into normal skin. In one instance the serum failed to sensitize the skin; in the second case mild sensitization occurred. The reaction was, however, much smaller than that obtained by sensitizing with unheated serum. The same serum heated for one-half hour at 56°C., while having less sensitizing strength than the unheated, sensitized more strongly than the serum heated at 56°C. for two hours. Serum taken from an individual sensitive to rabbit hair, when subjected to heat at 56°C. for 40

minutes, failed to sensitize the skin of three normal individuals. On the other hand, the effect of heat on serum taken from individuals sensitive to timothy pollen is clearly shown in the titration of the reaction with serum heated for different periods of time (Table II). The serum was divided into 8 portions: one, the unheated control; five, heated at 56°C. for 5, 10, 20, 30 and 60 minutes, respectively; two, heated at 38° to 40°C. for 30 and 60 minutes. The eight portions were injected into the skin of a normal individual, a fixed amount, 0.1 cc. of serum being given; 0.05 cc. of timothy extract, diluted 1 in 1000, (N = 46 mgm. per 100 cc.) was then injected into each sensitized site and the reactions were read after 15 minutes. We have reported (Chant and Gay) that normal serum heated at 56°C. for more than 20 minutes, and injected into normal homologous or heterologous skin, produces marked local reaction. The heating of serum taken from subjects sensitive to pollens, in like manner gives rise to reactions in heterologous skin. The maximal reaction occurred after the heating had been lengthened to one-half hour. These reactions had entirely subsided before the provoking timothy extract was injected. The transfer of hypersensitiveness was retarded even after 5 minutes of heat at 56°C., and aside from redness, after 20 minutes' heating the reactions observed were quite negligible. Heat at 38°C. to 40°C. for one-half hour, on the other hand, had the same effect as heat at 56°C. for five minutes; at 38° to 40° for one hour, the reactions resulting were equivalent to those after heat at 56°C. for ten minutes. The degree of heat, rather than the length of time the serum is heated, seems to be the influencing factor in diminishing the power to transfer passively hypersensitiveness.

Duration of passive sensitization. Although sensitization can be demonstrated for as long as 36 days after the injection of the sensitizing serum, nevertheless, there is apparently a gradual diminution in the reactive power of the sensitized area of skin. We were able to produce reactions in 5 cases 36 days after sensitization. The measurements of the reactions in these cases were considerably smaller, however, than those of the reactions obtained in the same cases 7 days after sensitization.

The effect of repeated injections of atopen into passively sensitized sites. Passive sensitization was demonstrated in the skin of thirteen

normal individuals. After a lapse of from 7 to 14 days the same group was reinjected in the same sites with a similar amount of pollen or dander extract. Of the thirteen, 8 cases failed to give any reactions. In five cases, however, reactions were obtained; without exception they were much smaller than the reactions following the first provoking injection of pollen extract. Theoretically, one might assume that a second reaction occurs only in those cases where there is present in the tissue an excessive amount of reagin which has not combined with the atopens of the first injection of timothy extract; further, that the second injection of timothy extract induces a reaction between the existing uncombined reagins and the added atopens. On the other hand, we have found that if a sensitized site is injected with a provoking atopen diluted 1 part in 100 parts of saline, the subsequent injection of a same dilution has no effect in the majority of instances. To produce a reaction, a solution 10 to 20 times more concentrated must be injected. Even then the reactions are not so marked as those which result from the initial injection.

The effect of combining serum from a sensitive individual with the specific extract before injection. The intradermal reactions following the injections of serum taken from an individual sensitive to timothy, mixed with timothy extract, termed for convenience Solution A, were compared with reactions following the injection of serum taken from an individual sensitive to timothy, combined with standard Coca solution and with normal saline. A number of control reactions were likewise observed; for example, the reaction following the injection of normal serum mixed with timothy extract was compared with the reaction from an injection of normal serum mixed with Coca solution and with saline. In the experiments the same proportions were maintained—two parts of serum with one part of diluent, timothy extract, saline and Coca solution being considered as the diluents. Each mixture was then diluted further, 1:100, with physiological saline solution and 0.1 cc. was injected in each instance. For comparison, corresponding sites were used on the right and left arms of the different normal individuals. In a few cases the mixtures were prepared at the time of injection, but in the majority of instances two hours elapsed between the time the mixture was made and the injection.

Measurements in Table III show that a mixture of serum from a

patient sensitive to timothy pollen and timothy extract, Solution A, produces a much greater skin reaction than a mixture of either the same serum mixed with Coca solution, or with saline. Both wheals and redness were more extensive. Comparison of serum from a patient sensitive to timothy pollen mixed with timothy extract to

TABLE III

Intradermal reactions of normal recipients to injections of sera taken from patients sensitive to timothy pollen mixed in vitro with timothy extract, and comparative measurements of the reactions resulting from the injection of control solutions

NORMAL RECIPIENTS	SOLUTION A		CONTROLS								TIME INTERVAL BETWEEN MIXING AND INJECTING. KEPT AT ROOM TEMPERATURE
	Timothy sensitive serum + timothy extract		Timothy sensitive serum + normal saline		Normal serum + timothy extract		Normal serum + normal saline		Timothy extract "control"		
	Wheal	Redness	Wheal	Redness	Wheal	Redness	Wheal	Redness	Wheal	Redness	
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	
M	1.7	4.5	0.9	2.0	1.1	2.0	0.9	2.0	0	0	2 hours
V	1.5	5.2	0.7	2.5	1.1	4.7	1.0	4.1	0.5	0.5	2 hours
F	1.9	7.4	0.9	5.6	1.0	1.5	1.1	1.4	1.0	1.0	1½ hours
E	1.7	6.0	1.2	4.5	1.3	3.7	—	—	0	0	2 hours
			Timothy sensitive serum + Coca'ssolution				Normal serum + Coca'ssolution				
Ha	1.1	4.5	1.0	1.1	1.1	1.7	1.0	1.7	0.8	1.0	40 minutes
Hb	1.5	4.8	1.0	1.2	1.1	1.7	1.0	1.7	0.8	1.0	1½ hours
W	1.3	4.4	1.0	1.2	0.9	1.5	0.9	2.0	0	0	45 minutes
K	1.1	5.7	0.8	0.9	1.0	2.5	1.1	1.8	1.1	1.1	2 hours
K	1.2	3.8	0.8	1.3	1.7*	5.5	—	—	0.8	1.5	2 hours
We	1.4	5.4	0.9	1.3	—	—	—	—	0.4	0	Immediate
Wb	1.3	5.0	1.0	1.1	—	—	—	—	0.4	0	1½ hours
Ew	1.3	3.8	0.8	0.9	—	—	—	—	0.9	0	2 hours
Fr	1.2	4.7	1.0	1.4	—	—	—	—	1.0	0	2 hours

* Solution two weeks old.

normal serum mixed with timothy extract, in eight cases, shows much greater reactions with the former than with the latter. The ninth case is an exception due to the fact that the normal serum was kept for two weeks in the ice-box; and as reported by us (Chant and Gay, 9) normal serum kept at ice-box temperature for 12 days or more is

irritating when injected intradermally. As a control, timothy extract diluted 1 in 100 was used in the series of cases and the reactions were negligible (Fig. 2).

Levine and Coca (7) have called attention to the large wheal and surrounding erythema resulting from the injection of such mixtures, but they found that the reactions from serum taken from individuals sensitive to timothy pollen mixed with timothy extract did not exceed the reactions from mixtures of such sera and physiological salt solution. Our findings are at variance with the work of these investigators. We also differ with Coca and Grove (8) and Prausnitz and Küstner, who have reported that mixtures of atopic serum and atopen

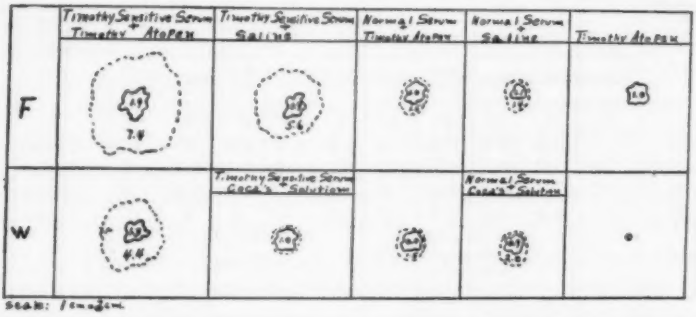

FIG. 2. REACTIONS FROM INJECTIONS OF SERUM TAKEN FROM PATIENTS SENSITIVE TO TIMOTHY MIXED WITH ATOPEN, SALINE AND COCA'S SOLUTIONS

fail to give definite reactions on injection. As a result of the experiment in 13 normal individuals we conclude that injections of mixtures of serum, from individuals sensitive to timothy, with timothy extract give intradermal reactions much larger than any of the combinations of controls. It is of interest to note that in one subject we compared the skin reaction following an injection of this mixture with the reaction obtained following an injection of timothy atopen into a site sensitized by a corresponding amount of serum. The results were quite similar. The mixture produced a wheal 1.9 cm. and redness 7.4 cm. in diameter; the injection of timothy into the sensitized site caused a wheal 2.0 cm. and redness 6.7 in diameter. Recently Clark and Gallagher (10) have concluded from their ex-

periments that "atopic reagins can be neutralized in a normal skin without producing the typical atopic reaction. The mechanism of the atopic reaction, as produced experimentally, must involve at least three substances, reagin, specific atopen and normal skin cells, and the reagin must have an opportunity to act upon the cell before coming in contact with the atopen." While we agree in part with these conclusions, nevertheless, the statement that the reagin must act upon the tissue cell before coming in contact with the atopen in order to give rise to the so called atopic skin reaction is not borne out by the experiments which we have presented. Our atopens and reagins were mixed *in vitro* and were then injected into the tissue cells with the resulting typical atopic reactions.

Further experiments are to be reported by us later concerning this phenomenon.

Contralateral passive transfer experiments with the serum of patients sensitive to timothy pollen, ragweed pollen, and rabbit hair. The phenomenon of passive transfer first described by Prausnitz and Küstner has been frequently confirmed. That is, the provoking atopen has been injected into the local sensitized sites of normal skin and marked specific reactions have appeared within fifteen minutes. In 1925, Freeman (11) demonstrated that local reaction occurred in sensitized mucous membrane or skin if the normal individual ate offending protein. He sensitized the mucous membrane of his own nose with serum of an individual sensitive to egg. He then ate a small quantity of egg and in a very short time experienced itching of the nose and some oedema with watery discharge. He likewise demonstrated a local reaction in the skin of a normal individual sensitized with the serum of a pollen-sensitive subject,—the reaction occurring after the injection (intravenous) of a small quantity of pollen extract. Lewis and Grant (12) have followed out Freeman's technique with somewhat similar results; and Walzer (13) has confirmed this direct method of demonstrating the absorption of incompletely digested protein from the digestive tract.

It seemed to us that the absorption of foreign protein from the skin into the general circulation would demonstrate in a more accurate way the above phenomenon. In the following experiment a normal individual was sensitized in three sites on the forearm, each 5 cm.

apart. After seven days 0.05 cc. timothy extract diluted 1 in 100 was injected into the middle site. Within 15 minutes a reaction appeared, characterized by both redness and wheals. The reaction at the upper site showed a wheal 3 by 3.5 cm. in area with redness 4 cm. in diameter. The reaction at the lower site was of the same intensity. We were able to demonstrate similar reactions in four normal individuals. The distances of the sites of sensitization from the provoking atopen injection varied from 5 cm. to 10 cm. both above and below the point of inoculation. This phenomenon suggested to us the possibility of contralateral passive transfer of hypersensitiveness; that is, the sensitization of a site or sites in the left arm and the injection of the provoking atopen in the normal skin of the right arm. We were able repeatedly to demonstrate this phenomenon in a number of normal individuals. Five patients are reported in Table IV. In two cases, one area was sensitized, in one case, five areas, in one case, three areas, and in one case two areas. The sensitizing serum was used both in concentrated form and in dilutions as high as 1 part in 64 parts of saline solution. In this series of tests

TABLE IV

Reactions in sensitized areas produced by timothy atopen injected into the opposite arm (contralateral passive transfer)

NORMAL RECIPIENT	CONCENTRATION OF SENSITIZING SERUM—INJECTED IN LEFT ARM	AMOUNT AND CONCENTRATION OF TIMOTHY ATOPEN—INJECTED IN RIGHT ARM	INTERVAL BETWEEN INJECTION AND APPEARANCE OF PAPULES	TIME REQUIRED FOR WHEAL TO ATTAIN MAXIMAL SIZE AFTER APPEARANCE OF PAPULES
G	1:2	0.05 cc. 1:1000 dilution	No reaction	No reaction
		0.05 cc. 1:100 dilution	No reaction	No reaction
		0.05 cc. 1:10 dilution	No reaction	No reaction
		0.1 cc. Concentrated	Reaction after 22 minutes	46 minutes
	1:4	0.1 cc. Concentrated	Reaction after 36 minutes	27 minutes
	1:8	0.1 cc. Concentrated	Reaction after 36 minutes	27 minutes
	1:16	0.1 cc. Concentrated	Reaction after 120 minutes	Not measured
	1:64	0.1 cc. Concentrated	Reaction after 120 minutes	Not measured
C	Undiluted serum	0.25 cc. Concentrated	Reaction after 10 minutes	20 minutes
S	1:2	0.1 cc. Concentrated	Reaction after 30 minutes	30 minutes
F	1:2	0.1 cc. Concentrated	Reaction after 22 minutes	48 minutes
W	1:2	0.1 cc. Concentrated	Reaction after 25 minutes	40 minutes

0.1 cc. of concentrated timothy extract was injected into the skin of the opposite arm, as we discovered that the contralateral injection of an extract diluted 1:1000, 1:100, or 1:10 failed to produce reactions in the sensitized sites. An analysis of Table IV shows that from 22 minutes to 2 hours after the injection of concentrated timothy in the right arm the patients showed reactions about the sensitized sites of the left arm. There was intense itching at these sensitized

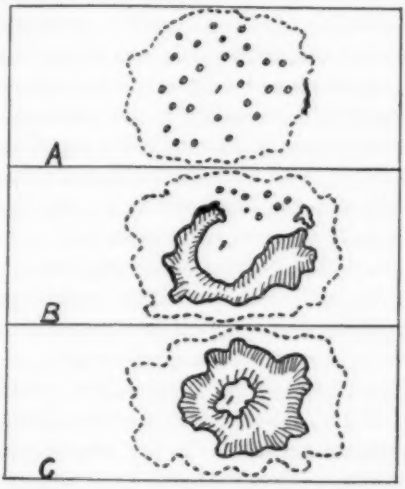

FIG. 3. DIAGRAMMATIC REPRESENTATION OF THE REACTIONS RESULTING IN CONTRALATERAL PASSIVE TRANSFER; (*a*) AFTER 30 MINUTES; (*b*) AFTER 45 MINUTES; (*c*) AFTER 75 MINUTES

areas. In one case we used undiluted serum and used as a provoking dose 0.25 cc. of concentrated timothy extract in the opposite arm. The reaction was almost immediate following this procedure. In another case, the serum was diluted 1 to 16 and 1 to 64. The reaction to the latter did not appear until two hours after the provoking injection of concentrated timothy. We have demonstrated, therefore, a contralateral reaction which depends principally on the concentration of the extract used as the provoking atopen, and to a less degree, on the dilution of the sensitizing serum.

Atopen which had been *diluted* failed to give a contralateral transfer reaction when an area in the opposite arm had been sensitized by *concentrated* serum. On the other hand, *concentrated* atopen produced a contralateral transfer reaction in an area on the opposite arm which had been sensitized with *diluted* serum, somewhat delayed, it is true, but equal to that which resulted in an area sensitized with undiluted serum. We have been able to demonstrate this phenomenon not only with serum from patients sensitive to timothy pollen but also with serum from patients sensitive to ragweed pollen and rabbit hair.

The appearance of this reaction is interesting because it differs strikingly from that obtained by direct passive transfer experiments. Within 30 minutes after the injection of the offending protein extract, in the majority of instances, numerous white papules appeared about the sensitized site (Fig. 3-A). There was a tendency for the papules to fuse into a crescent-shaped wheal 15 minutes later, and at the same time the entire area developed a pink flush (Fig. 3-B). In one hour a complete wheal had developed, with, however, a depression in the centre at the immediate site of the sensitizing injection (Fig. 3-C). This depression was apparent for several hours, when it was encroached upon by a large oedematous swelling; the reaction persisted thus for from 5 to 24 hours. Just as in the testing of an hypersensitive individual with the offending protein solution or as in direct passive transfer experiments, so in the contralateral experiments the patient complained of considerable local itching. In fact, this is one of the first sensations experienced with the appearance of the papules shortly after the injection of the foreign protein.

Since the beginning of our contralateral transfer studies, several very important differences from direct passive transfer experiments have been observed. First, if the serum of the hypersensitive patient is kept for more than four days, although direct passive transfer experiments could be demonstrated immediately, contralateral passive transfer could not be demonstrated until after the lapse of three hours. The reaction was somewhat less marked, however, than in the case where fresh serum was used.

Second: The ability to produce reactions by contralateral transfer depended on the so-called reagin content of the serum. Unless the patient from whom the serum was obtained showed a very marked

skin reaction on the direct injection of diluted foreign protein (1:1000), contralateral transfer failed, even though direct passive transfer showed very striking reactions.

Third: The clinical severity of the patient's symptoms seemed to indicate whether contralateral transfer could be demonstrated. We failed to bring about reactions in the skin of several normal individuals when we used serum of clinically mild sufferers from timothy hay-fever. This is quite the converse of our studies with direct passive transfer serum.

We have attempted to demonstrate the effect of heat on hypersensitive serum, and the relation of heat to contralateral transfer. The serum of a patient sensitive to ragweed was divided into three portions. One portion was used as the unheated control. A second portion was heated for 20 minutes at 56°C. A third portion was heated for 30 minutes at 56°C. The serum was injected into the skin of a normal individual according to the following scheme:

1. Left lower arm—unheated control serum was injected.
2. The left leg, thigh,—unheated control serum was injected.
3. The medial side of the upper left arm—serum heated 20 minutes at 56°C. was injected.
4. Outer surface of upper left arm,—serum heated 30 minutes at 56°C. was injected.

On the following day 0.1 cc. of concentrated ragweed extract was injected intradermally in the right forearm. Within five minutes papules appeared in the two areas sensitized with unheated control serum, and at the same time the individual complained of intense itching in these two areas. There was no difference in the time appearance of the reactions in the arm and leg, and as the reaction increased they seemed to parallel one another. Within 20 minutes a definite wheal was present surrounded by an area of redness and the wheal became gradually larger, following in every way the description as mentioned above. No reaction appeared in the two areas which had been theoretically sensitized with heated serum.

In order to check these reactions and to be certain that sensitization was not of a delayed type in the areas injected with heated serum, a second injection of concentrated ragweed was given on the following day in the right arm of the same individual. The result was

entirely negative. The sites which reacted on the previous day had apparently been desensitized by the contralateral reaction.

Attempts to sensitize guinea-pigs with rabbit-hair and horse-dander sensitive serum. Few investigators have succeeded in passively sensitizing guinea-pigs to the serum of sensitive patients. DeBesche was successful with horse serum but not with dander. Three groups of investigators, Grysez and Bernard (14), Achard and Flandin (15), and Longcope and Rackeman (16) were able to sensitize passively guinea-pigs with the serum of human beings who had been injected with therapeutic doses of horse serum and who had become sensitized to this serum. Schloss (17) describes two clear-cut cases in which he was able to sensitize guinea-pigs to egg and one case to milk by injecting the serum of infants specifically sensitive to these foods. Caulfeild (18) likewise reports a case (guinea-pig) in which slight sensitization was demonstrated (by the Dale technique) after passive transfer.

In our experiment virgin guinea-pigs weighing 130 to 230 grams were used, and the Dale method was selected as the most satisfactory in determining sensitization.

Three guinea-pigs were given intraperitoneal injections of 1, 2 and 5 cc. of serum from a patient sensitive to rabbit hair; one was given two injections of 1 cc. each with a 24-hour interval; two were given a series of 5 injections, each 0.5 cc., at 24-hour intervals. The Dale test was carried out after from 6 to 24 hours. There was no contraction of the uteri following the addition of rabbit-hair or horse-dander antigen to the bath. Each of the uteri, however, gave violent reactions when histamine was added to the bath.

To summarize the results of our experiments.

1. The sensitizing power of serum of hay-fever patients depends upon the reagin content of the serum, and not upon the severity of clinical symptoms. There is not sufficient evidence as yet to conclude that by our method of treatment we fail to "desensitize" our patients.

2. Heat at 56°C. rapidly diminishes the sensitizing power of the serum. The passive transfer of hypersensitive states could not be demonstrated if serum was heated for 40 minutes at 56°C. Heat at

38°–40°C. for 30 minutes fails to destroy completely the sensitizing power.

3. A site injected with sensitizing serum remained sensitive for at least 36 days, after which time the reactions became definitely smaller.

4. Second injections of atopen into sensitized areas produced in five cases a much diminished reaction, suggesting the possibility that reaction occurs only in the instances where the primary atopens fail to combine with the reagins existing in the sensitized tissue.

5. Reactions from the injection of mixtures of serum, from individuals sensitive to timothy pollen, with timothy extract are quite striking and much greater than from the combination of timothy extract with normal salt solution or the combination of serum, from individuals sensitive to timothy pollen, with normal salt solution, suggesting that neutralization does not become complete until after the injection into the normal tissue.

6. We have described the direct method of passive transfer as well as the phenomenon of contralateral passive transfer. The reaction of the latter phenomenon differs strikingly from the reaction obtained by the direct passive transfer technique. Contralateral transfer depends upon the concentration of the atopen; aged serum gives delayed reactions as compared with fresh serum; the reagin content of the serum must be high in order to sensitize the skin sufficiently to produce contralateral reaction, and the serum must be obtained from a patient who gives a history of severe symptoms of hay-fever or bronchial asthma. Heat at 56°C. for 20 minutes or longer retards the contralateral transfer phenomenon.

7. We were unable to sensitize guinea-pigs with serum taken from individuals sensitive to rabbit hair or horse dander.

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OBSERVATIONS ON THE BLOOD GUANIDINE IN TETANIA PARATHYREOPRIVA[1]

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The possible relationship between tetania parathyreopriva and guanidine intoxication has been suggested by the work of Noël Paton, Findlay, Burns, Sharpe and their co-workers. Noël Paton and Findlay (1) state that in tetania parathyreopriva "the symptoms are identical with those produced by the administration of salts of guanidine and methyl-guanidine." They also observe that "a comparison of the symptoms of guanidine poisoning with those of tetania parathyreopriva thus shows a very close correspondence. They bear the same relationship to those of tetania parathyreopriva that those following the administration of diphtheria toxin bear to the symptoms of true diphtheria."

In a series of experiments upon rabbits Noël Paton and Findlay (1) describe symptoms of tetany when guanidine hydrochloride in doses varying from 0.1 to 0.75 gram per kilo was employed. They also describe symptoms of tetany in cats when a dosage of 0.054 gram per kilo of guanidine hydrochloride was employed, and in a cat upon which a thyroparathyroidectomy was performed they noted that 0.03 gram per kilo of guanidine chloride produced symptoms, "a dose of guanidin," they add, "insufficient to produce symptoms in the normal animal."

By means of a colorimetric method we have found it possible to demonstrate methyl guanidine in the blood in amounts as small as 0.2 to 0.3 mgm. per 100 cc. of blood. We have applied this method to a study of the blood of a group of animals which were injected subcutaneously and intravenously with guanidine compounds in amounts sufficient to produce the tremors and nervous manifestations which Noël Paton and Findlay consider identical with those of tetania

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parathyreopriva. The animals employed in our experiments were dogs and rabbits, and the results are shown in Tables I, II, III, IV, V and VI. Methyl guanidine sulphate was used as the standard for the colorimetric comparison except in observations 1, 2, 3, 4, 5, and 6 of

TABLE I

Dog, male, weight 10 kilos

		BLOOD		
		N.P.N.	Urea N.	Methyl guanidine
9:15 a.m.		39	12.6	0.2
9:25 a.m.	1 gram methyl guanidine sulphate subcutaneously			
11:25 a.m.	No symptoms. Injection repeated in same dose			
1:00 p.m.	Dog frothing at mouth			
3:30 p.m.	Marked tremors, opisthotonus			
3:45 p.m.	Blood sample taken	63	36	2.86

Total dose 0.2 gram per kilo of body-weight.

TABLE II

Dog, female, weight 13.2 kilos

		BLOOD METHYL GUANIDINE PER 100 cc.
		mgm.
3:15 p.m.		0.2
3:20 p.m.	0.5 gram per kilo methyl guanidine sulphate injected into ileum	
3:27 p.m.	Tremors	1.55
3:34 p.m.	Tremors	2.80

Total dose 0.5 gram per kilo of body-weight.

Table VI, where guanidine carbonate was used. The calculations are in terms of methyl guanidine or guanidine as indicated.

The results of these observations indicate that when guanidine compounds are injected into animals in amounts sufficient to produce tremors and convulsions, a marked increase in the guanidine content of the blood can be determined. We also found that when animals

received a dose of guanidine insufficient to produce symptoms, an increase in the blood guanidine could still be determined, and that

TABLE III

Dog, male, weight 7 kilos

		BLOOD METHYL GUANIDINE PER 100 CC.
		mgm.
2:53 p.m.		0.2
2:55 p.m.	0.04 gram per kilo methyl guanidine sulphate intravenously	
2:59 p.m.		1.3
3:00 p.m.	0.08 gram per kilo	
3:03 p.m.	0.05 gram per kilo	
3:06 p.m.	No symptoms	1.2
3:07 p.m.	0.1 gram per kilo	
	Tremors	
3:15 p.m.		4.2

Total dose 0.27 gram per kilo of body-weight.

TABLE IV

Dog, male, weight 10 kilos

		BLOOD	
		N.P.N.	Methyl guanidine per 100 cc.
			mgm.
10:29 a.m.		24	0.2
10:32 a.m.	0.05 gram per kilo methyl guanidine sulphate intravenously		
10:33 a.m.		24	2.4
10:39 a.m.		26	1.5
10:44 a.m.		26.6	1.1
10:45 a.m.	0.05 gram per kilo		
10:49 a.m.	Tremors	30	2.2
10:54 a.m.		28.6	1.4
11:05 a.m.		26.6	1.2

Total dose 0.1 gram per kilo of body-weight.

doses as small as 0.01 gram per kilo of guanidine hydrochloride, when injected subcutaneously, produced a distinct rise in the blood guanidine.

Since we were able, by the colorimetric method, to demonstrate an increase in the guanidine in the blood when guanidine compounds were injected into animals, we applied this method to a study of the

TABLE V

Dog, male, weight 9 kilos

		BLOOD METHYL GUANIDINE PER 100 CC.
		mgm.
10:58 a.m.		0.2
11:01 a.m.	0.03 gram per kilo methyl guanidine sulphate intravenously	
11:06 a.m.		6.0
11:13 a.m.		4.6
11:23 a.m.	No symptoms	4.0

TABLE VI

RABBIT NUMBER	WEIGHT	DOSAGE	BLOOD GUANIDINE PER 100 CC. BLOOD		
			Before injection	20 minutes after	45 minutes after
	kilos				
1	3.5	0.01 gram per kilo guanidine hydrochloride	0.17	0.41	0.43
2	4.0	0.02 gram per kilo guanidine hydrochloride	0.21	0.44	0.55
3	3.8	0.03 gram per kilo guanidine hydrochloride	0.2	0.2	0.2
4	4.0	0.04 gram per kilo guanidine hydrochloride	0.19	0.47	0.25
5	3.4	0.06 gram per kilo guanidine hydrochloride	0.23	1.03	1.00
6	3.7	0.05 gram per kilo guanidine carbonate	0.3	0.57	0.54
7	4.2	0.02 gram per kilo methyl guanidine sulphate	0.3	0.40	0.42
8	3.8	0.05 gram per kilo methyl guanidine sulphate	0.23	0.33	1.10

None of the rabbits showed any symptoms of tetany.

blood of dogs in whom tetany had been produced by thyroparathyroidectomy.

Burns and Sharpe (2), by a method outlined in their paper, found the normal average amount of the blood guanidine in five dogs to be 0.1 mgm. per 100 cc., while in the blood of eight dogs on whom a parathyroidectomy had been performed the average amount was increased

to 0.87 mgm. per 100 cc. Noël Paton and Sharpe (3), by the use of the picric acid method, describe increases in the blood guanidine following parathyroidectomy. One dog showed, before parathyroidectomy,

TABLE VII

Dog, male, weight 14 kilos

Operated upon November 2, 1926. Thyroid and parathyroids removed. No food given for twenty-four hours before and twenty-four hours after the operation. Meat fed on each day thereafter. Blood taken every morning at 9 a.m. from the jugular vein, 30 cc., except as otherwise noted.

DATE	BLOOD CHEMISTRY				REMARKS
	N.P.N. per 100 cc.	Urea N. per 100 cc.	Guanidine per 100 cc.	Calcium per 100 cc.	
	mgm.	mgm.	mgm.	mgm.	
11/ 2/26	39.8	15.9	0.23	11.0	Operation at 1:30 p.m. Blood drawn at 9 a.m.
11/ 3/26	29.2	8.4	0.23	11.0	No food
11/ 4/26	28.0	9.8	0.23	9.6	Meat given hereafter daily in afternoon
11/ 5/26	37.5	18.2	0.23	7.8	No symptoms. Dog listless
11/ 6/26	41.4	17.52	0.46	7.4	No symptoms. Dog listless
11/ 7/26	41.0	17.28	0.37	7.0	No symptoms. Dog listless
11/ 8/26	55.0	28.9	0.40	5.6	In a.m. shows some signs of tetany. At 4 p.m. has tremors. Marked twitching of muscles. Blood taken also at 4:30 p.m.
4:30 p.m.	43.2	20.5	0.40	5.0	2.5 cc. Lilly's parathyroid extract (Collip) given. 50 units. At 9 p.m. tremors and twitching absent
11/ 9/26	33.5	9.30	0.21	8.0	No symptoms. Dog listless
11/10/26	30.8	13.1	0.23	8.4	No symptoms. Dog again lively and eager for food
11/11/26	34.3	17.8	0.21	7.4	No symptoms. Dog again lively and eager for food
11/13/26	45.5	27.6	0.34	9.0	Blood now drawn every other day. No symptoms
11/15/26	33.0	9.8	0.20	8.0	
11/18/26	32.0	8.0	0.20		
12/ 6/26	32.0	14.5	0.23	4.6	Tremors, weak, unsteady. Killed

1.92 mgm. of methyl guanidine per 100 cc. and after the development by tetany 3.85 mgm.; (on recrystallization, 1.20 and 2.65 mgm., respectively). The second animal showed 2.4 mgm. of methyl guani-

TABLE VIII

Dog, female, weight 16.7 kilos

Operated upon November 2, 1926. Thyroidparathyroidectomy. Same regime as before.

DATE	BLOOD CHEMISTRY				REMARKS
	N.P.N. per 100 cc.	Urea N. per 100 cc.	Guanidine per 100 cc.	Calcium per 100 cc.	
	mgm.	mgm.	mgm.	mgm.	
11/2/26	34.2	11.2	0.23	10.9	Operation at 2 p.m. Blood drawn at 9 a.m.
11/3/26	30.0	8.4	0.23	9.2	No food given
11/4/26	32.5	11.2	0.23	7.4	Meat given
11/5/26	41.5	20.6	0.23	7.2	Dog showing muscular twitching at 9 a.m.
12:30 p.m.	46.0	24.8	0.26	5.3	At 10:30 a.m. violent tetany. Dog has labored breathing Panting at 12:30 p.m. Gave 5 cc. Lilly's parathyroid extract (Collip). No relief. Dog died at 3:30 p.m.

TABLE IX

Dog, male, weight 13.3 kilos.

Operated November 8, 1926. Thyroidparathyroidectomy. Same regime as before.

DATE	BLOOD CHEMISTRY				REMARKS
	N.P.N. per 100 cc.	Urea N. per 100 cc.	Guanidine per 100 cc.	Calcium per 100 cc.	
	mgm.	mgm.	mgm.	mgm.	
11/8/26	30.0	8.4	0.22	8.4	Taken at 9 a.m. on day of operation. Operation at 1:30 p.m.
11/9/26	28.5	7.0	0.22	8.4	Food given in p.m.
11/10/26	38.0	14.3	0.22	6.8	Dog beginning to have tremors. Gave 2 cc. Lilly's parathyroid extract (Collip), 9 a.m.
11/11/26	35.4	14.9	0.23	10.6	No symptoms
11/13/26	48.5	28.5	0.23	7.2	No symptoms. Blood taken every other day
11/15/26	36.4	12.2	0.21	7.8	
11/17/26	30.0	7.5	0.22		
12/2/26	30.0	12.6	0.24	5.6	Violent tetany. Killed

TABLE X

Dog, male, weight 11.4 kilos

Operated November 8, 1926. Thyroidparathyroidectomy. Same regime as before.

DATE	BLOOD CHEMISTRY				REMARKS
	N.P.N. per 100 cc.	Urea N. per 100 cc.	Guanidine per 100 cc.	Calcium per 100 cc.	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
11/ 8/26	32.6	9.3	0.23	9.1	Blood drawn at 9 a.m. Operation at 2 p.m.
11/ 9/26	30.0	7.8	0.22	7.9	Began feeding in p.m. Meat diet
11/10/26	37.0	13.5	0.23	7.0	No symptoms
	40.0	18.6	0.34	6.2	Tetany at 4 p.m. 2 cc. Lilly's parathyroid extract (Collip) given. Blood drawn just before
11/11/26	38.5		0.23	11.2	No symptoms
11/13/26	55.0	30.8	0.23	9.0	No symptoms
					11/14/26 Tetany. 1 cc. Lilly's parathyroid extract (Collip) given. No blood taken
11/15/26	54.5	32.7	0.21	6.4	
11/17/26	66.5	38.0	0.30		Dog died during night. Blood from heart

TABLE XI

Dog, male, weight 12.2 kilos

Operated November 30, 1926. Thyroidparathyroidectomy. Same regime as before. Blood taken before operation and daily thereafter.

DATE	BLOOD CHEMISTRY				REMARKS
	N.P.N. per 100 cc.	Urea N. per 100 cc.	Guanidine per 100 cc.	Calcium per 100 cc.	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
11/30/26	24.0	9.8	0.20	10.8	Operation at 11 a.m. Blood drawn before operation
12/ 1/26	25.5	7.5	0.20	8.2	No food. No symptoms
12/ 2/26	25.5	8.9	0.20	7.8	Meat given. No symptoms
12/ 3/26	33.4	14.9	0.20	6.8	Slight tremors at 9 a.m. At 1:30 p.m. had violent spasms. Strong muscular twitching. Blood taken and dog killed
2 p.m.	32.0	15.9	0.21	6.6	

dine per 100 cc. before parathyroidectomy and 8.43 and 7.2 mgm. after operation; (on recrystallization, 1.68, 6.74, and 6.02 mgm., respectively).

The results of our observations are shown in Tables VII, VIII, IX, X, XI, and XII. Methyl guanidine sulphate was used as a standard for the colorimetric comparisons and the blood guanidine estimated in terms of guanidine.

A summary of these tables shows that in six dogs following parathyroidectomy with the development of tetany, we found a possible increase in the guanidine content of the blood in only one animal during one attack of tetany (Table VII). It later, however, had an attack

TABLE XII

Dog, male, weight 13.2 kilos

Operation January 4, 1927. Thyroidparathyroidectomy. Same regime as before.

DATE	BLOOD CHEMISTRY				REMARKS
	N.P.N. per 100 cc.	Urea N. per 100 cc.	Guanidine per 100 cc.	Calcium per 100 cc.	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
1/4/27	42.2	17.1	0.26	12.0	Operation at 11:30 a.m.
1/5/27	35.6	11.7	0.24	7.8	
1/6/27	44.2	17.3	0.24	6.0	Tremors
3:30 p.m.	43.6	17.8	0.37	6.2	Convulsions. Died

of tetany without any increase in the guanidine content of the blood. The first increase seemed definite but was, however, very slight when compared with the figures obtained by Noël Paton and Findlay, and by Noël Paton and Sharpe.

Because of the possibility that substances may be present in the blood of parathyroidectomized dogs that interfere with the colorimetric determination of guanidine, the blood removed from dogs during tetany was studied by the addition of known amounts of guanidine.

As stated previously (4) we are not sure that this method shows any guanidine in normal blood. The color produced is extremely slight and the amount when estimated by this method does not exceed 0.2

mgm. per 100 cc. In our readings on the blood of animals suffering from tetany to which 1 mgm. or less of methyl guanidine was added, the average recovery was from 50 to 75 per cent if we assume the normal to be 0.2 mgm. per 100 cc. If the value of the normal blood is actually zero, then the recovery was, on an average, from 80 to 90 per cent. The recovery of added guanidine was as great from the blood of dogs with tetany as for normal dogs.

We have also stated (4) that we have no proof that this colorimetric reaction is specific for guanidine. It can be produced, however, by the injection of guanidine into an animal, and if guanidine is present in the large amounts in the blood of animals in tetany, it should be detected by this method.

The findings in the blood urea nitrogen, and non-protein nitrogen following parathyroidectomy are in agreement with those of Haden and Orr (5), who described an increase in animals showing tetany. All of the animals following thyroid-parathyroidectomy showed a marked fall in the blood calcium, which occurred independently of any slight alterations in the blood guanidine.

SUMMARY

1. In animals in whom symptoms similar to those of tetany were produced by injections of guanidine compounds, an increase in the blood guanidine could be demonstrated by the colorimetric method.

2. Injections of guanidine compounds in doses insufficient to produce symptoms caused a demonstrable increase in the blood guanidine.

3. In five dogs suffering from tetany parathyreopriva, no increase in blood guanidine could be detected. In one dog with tetany parathyreopriva a slight increase in the color reaction occurred during one attack of tetany. In a later attack in the same dog no change was observed.

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THE SIGNIFICANCE OF THE H-ION CONCENTRATION FOR THE COLLOIDAL GOLD TEST[1]

A New Method for the Preparation of Colloidal Gold

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Although many modifications of the original Lange method for preparing colloidal gold have been described, every worker who frequently has to prepare gold sol must admit that none of these methods gives absolute satisfaction. On the one hand, the gold more or less frequently turns blue and cloudy and is obviously unfit for serological purposes; on the other hand, the sol may appear to be faultless but shows too high or too low a sensitivity, so that a tedious correction becomes necessary.

Briefly stated, the principle of the method of the preparation of colloidal gold consists in reducing the dissolved gold chloride to metallic colloidal gold of a cherry or ruby red color.

It is known that the preparation of the colloidal gold, as well as its sensitivity, is dependent on the acidity. Various methods have been described which take into account this fact; *e.g.*, Custer proposes first to neutralize the gold chloride solution in a preliminary titration with phenolphthalein as an indicator. The same procedure is followed by Haden. Ostwald neutralizes against litmus. Krebs and Ehmer, on the other hand, prepare several solutions with varying amounts of potassium carbonate and finally select the most suitable sol,—a somewhat tedious procedure.

All of these methods however seem to miss the two crucial questions which in my opinion can be formulated thus: (1) which is the *optimal pH* for the reduction of the gold? (2) Which is the *optimal pH* for the colloidal gold test with the spinal fluid?

By numerous measurements (our determinations were made colori-

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metrically, with the LaMotte apparatus), we were able to answer the second question by ascertaining that a "correct" colloidal gold should have a pH of 6.1 to 6.3; Mayr had found an optimal pH of 6.0 to 6.1. There remained the first question to be answered; with this answer we hoped to avoid failures in preparing the colloidal gold, and furthermore to obtain directly a colloidal gold of a pH of 6.1 to 6.3 and thus avoid a later tedious correction by adding alkali or acid.

As we have seen, Custer "neutralizes" against phenolphthalein and Ostwald against litmus. According to the nature of these indicators that means that Custer reduces the gold at a pH of 8.3 to 8.9, Ostwald at a pH between 9.0 and 9.6; this high alkalinity explains the frequency of failures as well as the low sensitivity of the final gold sol.

Our experiments show that if the gold chloride was reduced at a pH of 7.2 to 7.6—near the true neutral point—one finally obtains a correct colloidal gold of pH 6.1 to 6.3.

In order to be able to work at this pH it is essential to use an indicator with a sharp change within this range and as such we found phenol red to be the most suitable.

But although we were now working at a much more constant acidity, we still failed to produce a correct solution with absolute regularity. At this point, our attention was drawn[2] to a statement of Ostwald that, by using tannic acid as the reducing agent, ruby red gold sol could be obtained with absolute regularity. According to Ostwald this is due to the fact that the tannic acid acts not merely as a reducing agent but also as a protective colloid. We were able to confirm Ostwald's results, but the gold sol thus obtained was unfit for serological purposes because it was not sensitive enough. This lack of sensitivity apparently was caused mainly by the protective action of the tannic acid and not by the alkalinity of the gold sol, because even with the addition of acid the sol could not be rendered sensitive enough.

We succeeded in making use of the protective action of the tannic acid in the preparation of colloidal gold and eliminating the prohibitive action on the gold test with the spinal fluid by adding tannic acid

[2] By Dr. B. Wuth, New York.

as protective colloid in very small amounts and by using formaldehyde as the chief reducing agent.

The mode of procedure is as follows:

Reagents

1 per cent gold chloride (Merck)
3 per cent potassium carbonate
2.5 per cent formaldehyde (prepared from Merck's 37 per cent Formaldehyde)
0.1 per cent tannic acid
0.4 per cent Phenol Red (alcoholic)

TABLE I

	SOL	REDUCING pH	FINAL pH	REACTION OF SOL WITH SPINAL FLUID	
				Positive fluid	Negative fluid
Correct sol.	30	7.2	6.2	5555552100	0011000000
	31	7.2	6.2	4+55554+1+00	0000000000
	32	7.1	6.2	5555543210	—
	33	7.2	6.2	4555554+210	0111100000
	34	7.2	—	4555521000	0011000000
	35	7.2	—	555554+2+100	—
	36	7.2	—	4555553+210	—
	37	7.2	6.2	5555542+100	0011000000
	38	7.2	6.3	5555553210	—
	39	7.2	6.2	55555431+10	0001110000
	40	7.2	6.1	555532+1100	0000000000
	42	7.2	6.1	555553+32+10	0001110000
	43	7.1	6.2	55553+32100	0011100000
	44	7.2	6.3	5554+52100	0000000000
	45	7.1	6.3	45555542+10	0001100000
	46	7.0	6.2	4+555554320	0001100000
Too active sol.	47	6.6	5.0	5555555555	124+4+32100
	48	7.0	5.8	5555553+100	—
	49	6.8	5.0	5555555554	123+3+3+11100
	50	6.8	5.8	4+55555541+0	0012210000
Underactive sol.	51	8.0	6.6	2+555554210	0000000000
	52	7.3	6.8	24+5543200	0000000000
	53	7.6	6.6	255553+2+100	—
	54	7.5	—	2155554+100	0000000000

Chemicals should be pure and glassware perfectly clean.

Preliminary titration. To 100 cc. distilled water add 1 cc. of gold

TABLE II

SOL	FRESH SOL			pH OF SOL AND REACTION WITH SPINAL FLUID																				
				Day																				
	Reducing pH	Final pH	Curve with paretic and negative spinal fluid	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	Curve with paretic fluid	90th	Curve with paretic fluid
11	7.2	6.2	5555542+100 0011000000					6.3		6.3	6.3	6.3	6.3		6.3	6.4	6.5			6.5	2345521100		
15	7.2	6.2	55555431+10 0001110000					6.2		6.2	6.2	6.1	6.1		6.2	6.2	6.2			6.3	4555553210		
16	7.2	6.2	55554+53100 —					6.2		6.2	6.2	6.2	6.2		6.3	6.3	6.3			6.5	4+55554+3210		
18	7.2	6.1	555532+1100 0000000000					6.1		6.1	6.1	6.1	6.1		6.3	6.3	6.4			6.4	244+54+21+00		
19	7.2	6.1	555553+32+10 0001110000					6.1		6.1	6.1	6.1	6.1		6.1	6.2	6.2			6.4	255553+1+000		
20	7.1	6.2	555543+32100 0011100000					6.0		5.9	6.0	6.0	6.0		6.2	6.2	6.2			6.3	2+555543+100		
21	7.2	6.2	5554+521000 0000000000					6.0		6.0	6.0	6.0	6.0		6.2	6.2	6.2			6.3	555532100		
13	7.4	6.8	22+34443200 0000000000					7.0		7.0	7.0	7.0	7.0		7.0	7 0	7.2			7.3	0012221+1+00		

25	—	6.5	4+555532100			6.5	7.3												2221100000
26	—	6.5	4+555532100			6.5	7.1												0000000000
27	—	6.5	4+555532100			6.5	7.3												4322+100000
29	—	6.5	4+555421100	6.5	6.5								7.3	4+55432100					
22	7.2	6.2	5555553210 0011000000	6.2	6.2	6.2							6.3	4555553+2+10					
24	7.1	6.2	555554+4310 0000000000	6.2	6.2	6.2							6.2	55553+21+100					

chloride solution and 2 drops of Phenol Red indicator; then add potassium carbonate solution until a definite pink remains from 1 to 1½ minutes (usually 0.5 cc.).

Preparation of colloidal gold. Place 250 cc. distilled water over bunsen flame; add 2.5 cc. gold chloride and an amount of potassium carbonate calculated in the previous titration; after shaking, add 1 drop of 0.1 per cent tannic acid. Heat until solution boils vigorously. Remove flame. Add 1.5 cc. formaldehyde quickly and shake continuously until solution turns a clear deep red. The temperature should be kept close to boiling point until the final color change has taken place. When the solution has cooled to room temperature, it should be tested with a known paretic and with a normal spinal fluid.

Using this method, we have been able to obtain correct colloidal gold with absolute regularity; we prepared about fifty different sols; even taking reagents from different firms did not influence the result if only the neutralization was performed exactly.

This enabled us to study the influence of the pH on the colloidal gold tests with the spinal fluid. Table I shows the close relationship between the pH of the sol and its sensitivity. Even a slight excess in acidity renders the sol too sensitive, whereas a slight excess of alkalinity makes it underactive. This proves that Mayr was wrong when he claimed that the hydrogen-ion concentration was of influence only when massive alterations in pH occurred.

As it is known that all colloids show a change in the process of "aging," it seemed of interest to examine gold sols which had been kept for some length of time. This is important because, in Europe especially, colloidal gold is being placed on the market by chemical firms.

Table II demonstrates several sols that were examined during and after varying periods of time. It is seen that in course of time the sol becomes more alkaline and decidedly less active; according to these results it does not seem advisable, for the colloidal gold test, to use a sol that is older than eight to ten days.

SUMMARY

A new method for the preparation of colloidal gold has been described which is based on the consideration of the hydrogen-ion con-

centration and the addition of a protective colloid. The influence of the hydrogen-ion concentration on the preparation of the colloidal gold and on the colloidal gold test has been demonstrated. It has been shown that colloidal gold in "aging" becomes more alkaline and less active and that therefore for the colloidal gold test colloidal gold should be freshly prepared.

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THE EFFECT OF THYROID FEEDING ON THE SPONTANEOUS ACTIVITY OF THE ALBINO RAT AND ITS RELATION TO ACCOMPANYING PHYSIOLOGICAL CHANGES[1]

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For some time we have been studying the factors involved in the modification and control of the spontaneous motility of organisms. In the work thus far, concerned chiefly with the activity of the albino rat as measured in a revolving drum, it has proved a relatively simple task to *depress the activity*. Removal of ovaries or testes produces a drop of 65 to 95 per cent in the running average. Pregnancy and pseudo-pregnancy have a similar effect. Adrenalectomy produces a marked permanent depression, and spontaneous illness causes an immediate decrease. It has been a very difficult task, on the other hand, to *increase the activity*. This result was achieved with the spayed and castrated animals mentioned above, when it was found that the constant low level of activity might be increased to its original normal level by transplantation of ovaries or testes to these animals. All other means of stimulation, however, have failed. Alcohol, various drugs, pituitary extract, all given by mouth, have had no effect on the activity of otherwise normal animals. It occurred to us that thyroid feeding, which speeds up body metabolism, might also increase spontaneous activity. It is a matter of current opinion that hyperthyroid patients are overactive. Are hyperthyroid rats more active than normal animals? The results of experiments performed to answer this question are presented below.

Besides the motility change, we have studied also the accompanying physiological modifications expressed by body-weight, body-temperature, pulse rate, and food and water intake. From these observations we have hoped to determine whether these physiological modifications

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bear to the activity change any constant relationship by which that change might be explained.

METHODS

The rats were placed in individual activity cages (fully described by Richter and Wang, (12)), which had two compartments, a very small living cage, and a revolving drum to which the animal had free access at all times. The axle of the drum was connected by a series of levers to a cyclometer, which registered the turning of the drum both clockwise and counter-clockwise, so that the daily cyclometer reading gave a measurement of the activity of the animal. The living cage contained a food-cup and a graduated water bottle. At noon every day the cyclometers were read, the food-cups weighed and replenished, and the water bottles read and refilled. These records of food and water consumption represent indirectly the total metabolism of the animals. Body-weight, body-temperature, and electrocardiograms were taken once a week. The details of the general experimental procedure and control of environmental conditions, presented in previous papers from this laboratory (10, 15, 17), need not be repeated here.

Sixteen rats 46 days old, were used in these experiments. Seven males and seven females were litter mates; the other two, one of each sex, were selected from another litter born on the same day. These sixteen animals were equally divided into four groups (two males and two females in each group), and fed thyroid gland as follows:

GROUPS		FROM 46 TO 108 DAYS	FROM 108 TO 150 DAYS
I		Normal food	4 grains of desiccated thyroid gland in 100 grams of food
II	a	2 grains of desiccated thyroid gland in 100 grams of food	Normal food
	b	4 grains of desiccated thyroid gland in 100 grams of food	Normal food
	c	8 grains of desiccated thyroid gland in 100 grams of food	Normal food

The desiccated thyroid gland used was manufactured by Armour and Company in tablet form. These tablets were ground into fine

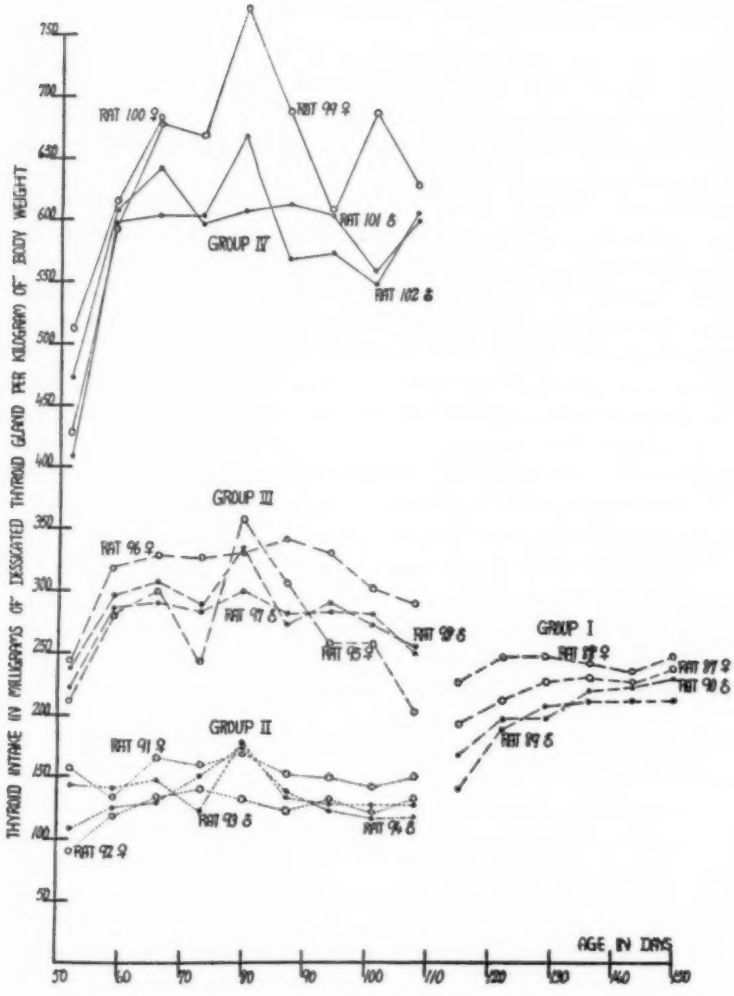
THYROID INTAKE IN MILLIGRAMS OF DESSICATED THYROID GLAND PER KILOGRAM OF BODY WEIGHT
AGE IN DAYS
GROUP IV
RAT 100 ♀
RAT 99 ♀
RAT 101 ♂
RAT 102 ♂
GROUP III
RAT 96 ♀
RAT 97 ♂
RAT 98 ♂
RAT 95 ♀
GROUP II
RAT 91 ♀
RAT 93 ♂
RAT 96 ♂
RAT 92 ♀
GROUP I
RAT 97 ♀
RAT 98 ♂
RAT 99 ♂

FIG. 1

powder and thoroughly mixed with a standard diet recommended by Dr. E. V. McCollum, containing the following constituents:

	per cent
Whole Wheat Flour	72.5
Skimmed Milk Powder	10.0
Casein	10.0
Butter Fat	5.0
Calcium Carbonate	1.5
Sodium Chloride	1.0

The amount of thyroid which each animal received during the period of experimental feeding depended entirely on its daily food intake. Figure 1 shows the variations of daily thyroid intake in milligrams of desiccated thyroid gland per kilogram of body-weight for the individuals of the four groups.

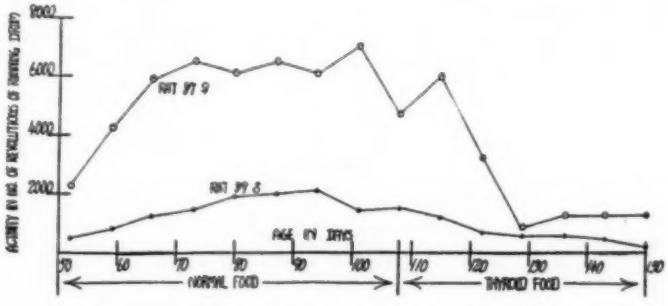

FIG 2a

RESULTS

A. Activity changes. Thyroid feeding was found to have a definite depressive effect on the activity of the rat. This is quite well demonstrated by the individual activity curves given in Figure 2a. These curves were obtained from one male and one female which received no desiccated thyroid gland until after 108 days of normal diet. In both cases there was a decrease in activity about two weeks after the thyroid feeding was begun. All four animals of the group gave a similar result. The average amount of daily activity during the period of normal diet was 2400 revolutions for the two males, and 6800 for the two females.

Two weeks after the experimental diet was started, the running averages of the two males dropped to 650 revolutions, and those of the two females to 1600. This is a 75 per cent decrease in activity.

The results given by the second group confirmed this fact in another

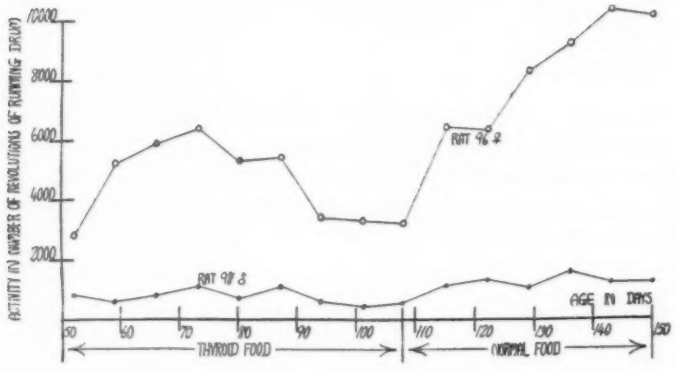

FIG. 2b

TABLE I

GROUP	46–108 DAYS OF AGE		108–150 DAYS OF AGE	
	Period of	Running average in number of revolutions of the drum	Period of	Running average in number of revolutions of the drum
A. Male rats				
I	Normal food	2,400	Thyroid food	700
II	Thyroid food	1,100	Normal food	1,400
B. Female rats				
I	Normal food	6,800	Thyroid food	3,100
II	Thyroid food	2,300	Normal food	4,900

way. These animals were fed thyroid from 46 to 108 days of age, and then returned to normal food. The activity level was low during the earlier period, but showed a marked rise after the thyroid diet was discontinued (see Fig. 2b). The running averages increased during

the final period of normal feeding from 1100 revolutions in the males and 2300 in the females to 1400 revolutions in the males and 5600 in the females. These averages show an increase in activity of 27 per cent for the males and 143 per cent for the females. These results

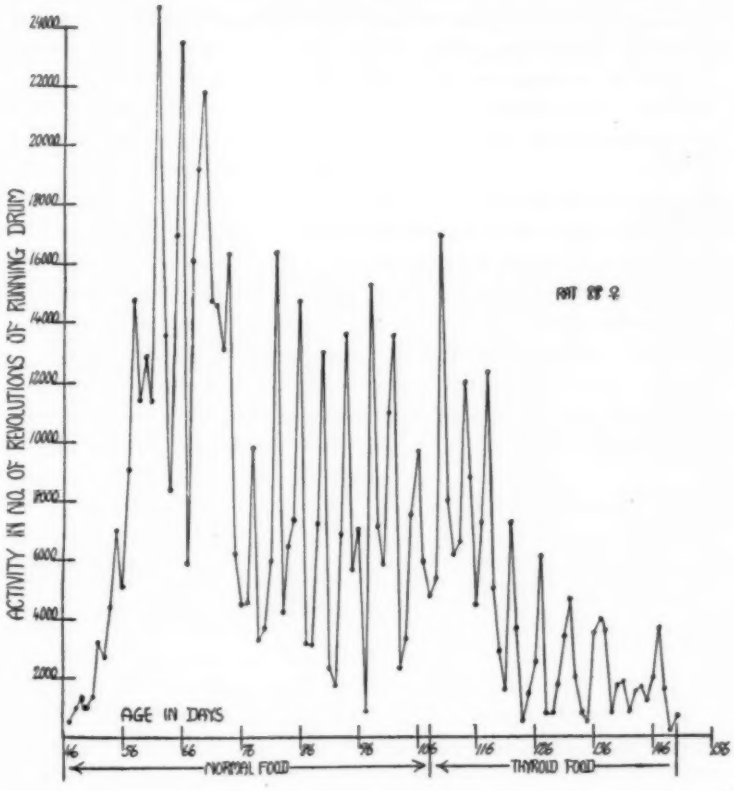

FIG. 2c

demonstrate plainly that the activity is decreased during thyroid feeding and rises after its cessation.

This depressive effect of thyroid administration on activity can be demonstrated in still another way. Table I, A and B, presents a com-

parison of the running averages of the animals of Groups I and II throughout the whole experiment. This table shows clearly that, during each period, the thyroid-fed rats had a lower activity level than those receiving normal food. Furthermore, it must be pointed out that thyroid feeding reduced greatly the sudden outburst in activity at puberty as described by Slonaker (13), Richter (9), Wang (15), and Hitchcock (7) (compare Figs. 2c and d).

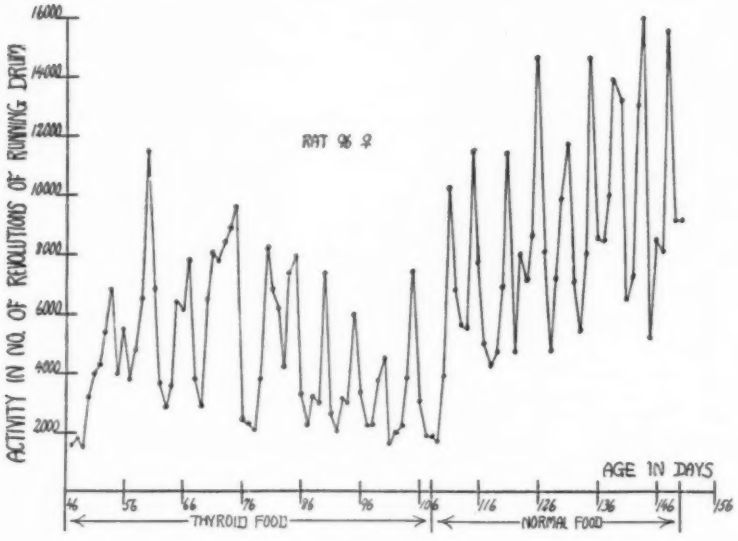

FIG. 2d

It is a noteworthy fact that, although the daily running averages were greatly reduced, the thyroid-fed female rats still showed the oestrous rhythm in the daily fluctuations of activity as described by Wang (15), Slonaker (14), Durrant (4), Hitchcock, and Bugbee and Simond (1). An activity curve of Rat 88 from Group I is given in Figure 2c, and one of Rat 96 from Group II in Figure 2d. From these curves we see that during the period of thyroid administration the activity of these female rats was low, but still showed the four-day rhythm. It will be observed, on close inspection, that during the period of thyroid feeding the activity rhythm was slightly longer than

during the time of normal feeding. For the six female rats of Group II, the average length of the cycle was 5.2 days throughout the period of experimental diet, and 4.3 days during the period of normal food. For the two females of Group I it was 4 days throughout the normal feeding and 5.2 days during the period of thyroid administration. This lengthening of the activity rhythm is, no doubt, a direct effect of thyroid consumption on the oestrous cycle. Cameron and Amies (2) found that experimental hyperthyroidism prolongs the oestrous rhythm in the mouse, expecially the stage of cornified epithelial cells. This is confirmed by our present findings on the rat, from simultaneous observations of activity and vaginal smears.

B. Physiological effects. We recorded further physiological changes produced by thyroid administration, changes in body-weight, food and water consumption, pulse rate, and body-temperature. It was noticed, in agreement with the observations of former investigators (3, 5, 8), that the body-weight of our experimental animals was generally lower than that of the normals, but showed a distinct rise after the thyroid diet was discontinued. This change in the body-weight is the only one of the physiological effects of thyroid feeding which runs parallel with the change in activity. All the others give an opposite picture of the activity change.

In the thyroid-fed rats, the food intake was greatly increased. This increase can be measured either absolutely in the number of grams of food consumed daily, or in the number of calories per kilogram of body-weight. Since these animals generally weigh less than normals, however, the true magnitude of the increase in food intake during thyroid administration can be ascertained only by a study of food intake measured in calories per kilogram of body-weight. The weekly averages of daily food intake for each animal are calculated in calories per kilogram of body-weight according to the following formula, given also in a previous paper (16).

$$C = \frac{F \times 1000}{\frac{W_1 + W_2}{2}}$$

where C = the weekly average of daily food intake in calories per kilogram of body-weight.

F = the weekly average of daily food intake in calories.
W_1 = the body-weight of the animal at the beginning of the week.
W_2 = the body-weight at the end of the week.

Figure 3a gives the individual food intake curves in calories per kilogram of body-weight for one male and one female rat of Group I, fed thyroid after 108 days of age. In both cases there was a distinct increase in food intake after the thyroid feeding was started. In Figure 3b, the individual food-intake curves for one male and one

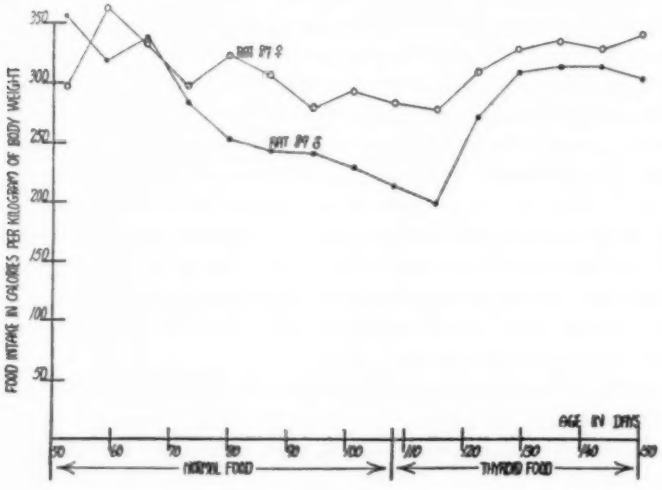

FIG. 3a

female of Group II, started on thyroid and then later given normal food, are presented. These curves demonstrate clearly that the food intake increased markedly during the thyroid feeding and returned to the normal level afterwards.

Thyroid feeding produces the same changes in the daily water intake as in the daily food consumption. On account of the difference in body-weight between the thyroid-fed and normal rats, it is necessary here also to compare the grams of water consumed per kilogram of body-weight. This is calculated according to the same principle as that used in the case of the food intake. Figure 4 (a and b) presents

some typical curves. It will be observed that thyroid feeding produced a very large increase in water intake. Under these conditions a rat may drink daily a quantity of water equal to one-quarter or one-third of its body-weight. This increase, too, is consistently larger in the male than in the female. One of the males used in these experiments, for instance, which weighed 225 grams, drank in one day 116

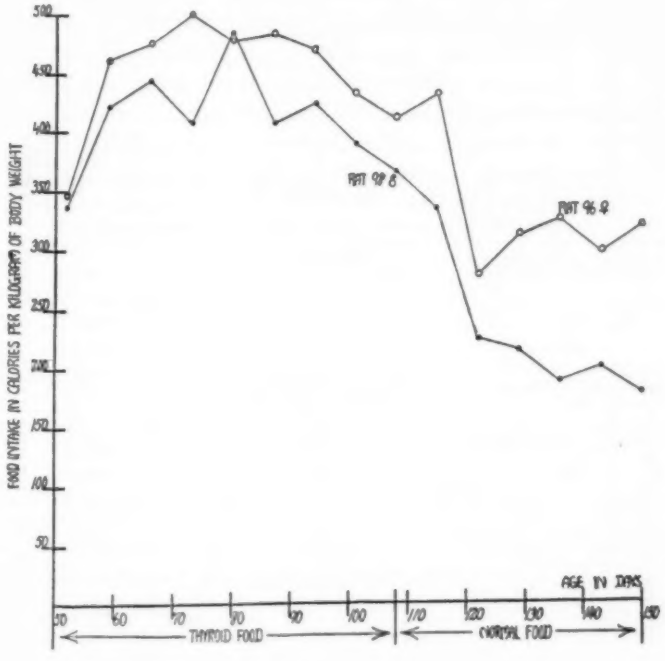

FIG. 3b

grams of water. This large water consumption was present only so long as thyroid was administered. After the return to normal food, the water intake invariably decreased, within a few days, to the normal level.

Besides the increase in food and water intake, we observed an increased heart rate and body temperature in the thyroid-fed rats.

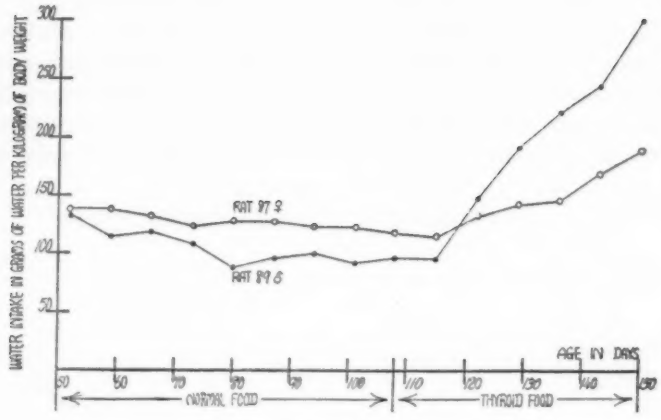
WATER INTAKE IN GRAMS OF WATER PER KILOGRAM OF BODY WEIGHT
300
250
200
150
100
50
RAT 97 ♀
RAT 89 ♂
AGE IN DAYS
50
60
70
80
90
100
110
120
130
140
150
NORMAL FOOD
THYROID FOOD

FIG. 4a

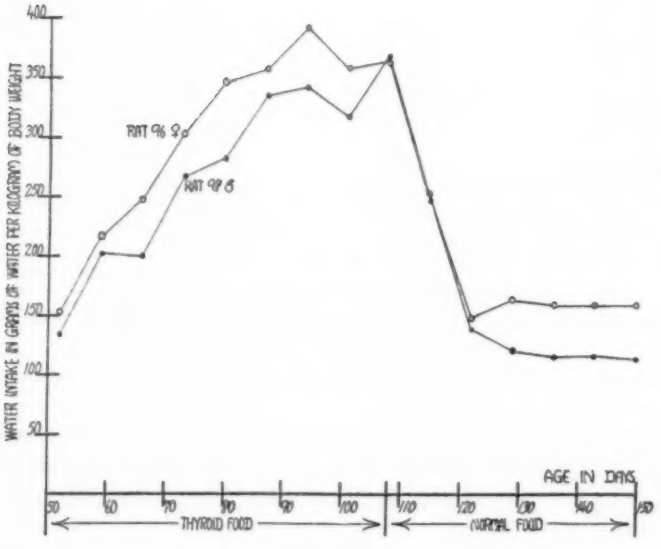
WATER INTAKE IN GRAMS OF WATER PER KILOGRAM OF BODY WEIGHT
400
350
300
250
200
150
100
50
RAT 96 ♀
RAT 98 ♂
AGE IN DAYS
50
60
70
80
90
100
110
120
130
140
150
THYROID FOOD
NORMAL FOOD

FIG. 4b

The pulse rate was determined from electrocardiograms taken once a week on all the rats under experimentation, according to a method devised by Richter (11). The backs of the right front-foot and the left hind-foot were washed with alcohol and ether and scratched with a sewing-needle to eliminate the high skin resistance. Then, electrodes, made of small pieces of sheet zinc and a paste of kaolin and saturated zinc sulphate solution, were applied over the prepared areas. An assistant wearing rubber gloves kept these electrodes in place. A Hindle string galvanometer was used, with the tension of the string set at one centimeter per one millivolt with the rat in circuit. This investigation showed that the heart rate of a normal rat averages about 560 beats per minute, that thyroid feeding increases the rate to an

TABLE II

Showing the effect of thyroid feeding on the pulse rate of the rat

GROUP I	AVERAGE PULSE RATE DURING PERIOD OF NORMAL FEEDING	AVERAGE PULSE RATE DURING PERIOD OF THYROID FEEDING
Rat 89	583	636
Rat 87	566	680
GROUP II	**AVERAGE PULSE RATE DURING PERIOD OF THYROID FEEDING**	**AVERAGE PULSE RATE DURING PERIOD OF NORMAL FEEDING**
Rat 98	680	486
Rat 96	643	513

average of 660, and that there seems to be a decrease in heart rate below the normal level after thyroid feeding is discontinued (Table II).

Body temperature was determined by a one-minute clinical thermometer introduced into the rectum of the males and the vagina of the females. It was found that the average body temperature of a normal rat, 99.1°F. was increased by thyroid feeding to 100.0°F. and that the body temperature of the thyroid-fed rats returned to the normal level after the thyroid administration was discontinued. These readings are suggestive, but not quantitative, because of the difficulty of keeping the animal quiet while the temperature is being taken.

The same changes in activity, body-weight, food and water intake, pulse rate and body temperature were obtained in all the sixteen rats and with each of the doses of desiccated thyroid gland. Owing to the

large individual variation in the susceptibility to the thyroid feeding and the small number of animals used, the only difference in dosage effect noticed was that two males and one female from the group receiving the heaviest dose, and one male from the group taking the next largest dose died. It seems from this that the males are more sensitive to thyroid than the females.

SUMMARY AND DISCUSSION

1. In view of the increase in metabolism which thyroid extract is known to produce, it seems probable that hyperthyroidism might be accompanied by an increase in gross bodily activity. The experiments reported above, however, have demonstrated conclusively that desiccated thyroid gland, fed by mouth, produces a marked decrease in the spontaneous running activity of the albino rat. The activity level remains low as long as the thyroid extract is administered, but increases to normal very quickly after the experimental diet is discontinued.

2. The metabolic changes in our hyperthyroid rats were expressed in an increased food and water intake, increased pulse rate and body temperature, and decreased body-weight. An acceleration of the metabolic rate always means that the animal body is dissipating energy more rapidly than it does normally, and consequently requires more food; and the extra heat generated in the oxidation process tends to increase the evaporation of water from the skin, thus drying the tissues and stimulating the animal to drink more water.

The excess of energy consumed by the animal seems to be used entirely in the oxidation process, so that none is left for greater mechanical activity. This fact, however, suggests only a possible explanation as to why the motility is not increased; it tells us nothing of how the running average is actually so greatly decreased. Two explanations of this phenomenon may be offered at the present time. The increased metabolism produced by the thyroid feeding may injure the tissues and thus depress the activity. We know definitely that any illness affects the activity of the rat more readily than any other phase of its behavior. The second explanation, however, seems more likely. The thyroid feeding may render the animal more susceptible to fatigue. A. V. Hill (6) and others have suggested that an animal's

ability to perform muscular work is limited by the pumping power of the heart. Now, in the case of the hyperthyroid rats, the greater metabolism requires that the heart circulate the blood more rapidly in order to supply sufficient oxygen for the burning process. Muscular activity further increases these demands, so that a stage is soon reached at which more oxygen is required than the heart can supply, the waste products which we know produce fatigue accumulate in the body, and the animal is unable to run.

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(15) WANG: *Comparative Psychology Monographs*, 2, 1923, no. 6.
(16) *Idem:* Amer. Jour. Physiol., 1925, lxxi, 729.
(17) WANG, RICHTER AND GUTTMACHER: *Ibid.*, 1925, lxxiii, 581.

TECHNICAL DIFFICULTIES ENCOUNTERED IN AN ATTEMPT TO PRODUCE BACTERIAL MUTATIONS[1]

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According to d'Herelle (1) and others, mutations are of common occurrence among bacterial species and may be induced by certain procedures. In such studies, precautions against the possibility of contamination cannot receive too great attention. Furthermore, reliance upon filtration to remove bacteria is fraught with danger. Our knowledge of the factors which render organisms filtrable or non-filtrable is still far from complete.

The technical simplicity of the procedures and the startling results recently reported by Pryor (2) led us to attempt a repetition of some of his experiments.

It is stated that, as a result of various conditions, such as growth in contact with body fluids, rapid serial transplantation, variation in substrate, and others, changes may be induced in the causative agent of scarlet fever. One of the changes described is a mutation from Streptococcus scarlatinae, by means of certain subcultures, into Staphylococcus aureus or albus and from this, under the influence of bacteriophage, into a filtrable form which, in turn, after passing through a filter and without further treatment, will change into an organism morphologically indistinguishable from B. diphtheriae.[2]

A series of procedures is outlined by which the change from Streptococcus scarlatinae into a spore-forming coccus may be brought about. We have attempted to induce this transformation by employing the technique outlined by Pryor.

A recently isolated culture of streptococci from scarlet fever is plated out massively on a rabbit-blood-agar plate. After 24 hours'

[1] Received for publication March 14, 1927.

[2] It may be pointed out that some strains of streptococci, under the influence of cultural conditions may assume, temporarily, forms which are quite diphtheroid. Careful examination, however, will usually enable one to make the distinction.

incubation at 37°C. the growth is transferred *en masse* to slants of 1.5 per cent agar containing 1 per cent peptone and 10 per cent sucrose. After incubation for 24 hours at 37°C., it is said, staphylococci will have developed which can be shown to be of a type either resistant or sensitive to bacteriophage, and the resistant form may be either of the albus or aureus varieties. After making dilution-pour-plates of the sensitive type, and incubating for 24 hours at 37°C. and then for an indefinite time at room temperature, it is said there will appear colonies of a spore-forming coccus or cocco-bacillus, called *Sporococcus*.

Following the procedure outlined above we carefully inoculated the entire surface of a rabbit-blood-agar plate with a strain of streptococci believed to be Streptococcus scarlatinae isolated by one of us from a case of scarlet fever and shown to produce a characteristic Dick reaction. This was incubated for 24 hours at 37°C. and then carefully examined for evidences of contamination, or mutation. No visible contamination or mutation having been found, the growth from the plate was scraped off and transferred, *en masse*, to two slants of the sucrose-agar mentioned above and also to one blood-agar slant. These slants were then incubated at 37°C. for 24 hours. At the end of this time all three slants were found to have a mixed growth of staphylococci and streptococci, thus far seeming to confirm the results of Pryor.

In view of the possibility that staphylococci may have been present but hidden in the massive growth on the original plates, *i.e.*, prematurely in the prescribed program, and therefore possibly contamination, it was thought advisable to take greater precautions against this error. For this reason a Kolle flask and a plate were prepared with rabbit-blood-agar and incubated for 24 hours at 37°C. to test sterility. Being found sterile, they were inoculated simultaneously in a special, almost dust-free, cabinet with a repurified culture of the streptococcus mentioned above. After incubation the growth on the plate was found to contain, besides the streptococci, three small colonies of staphylococci. The growth in the Kolle flask appeared, upon examination with a jeweler's lens, to be free from staphylococci.

From the plate two sucrose-agar slants were heavily inoculated, one (a) with material from the half of the plate containing the staphylococcus colonies and the other (b) with material from the other half of the plate. From the Kolle flask, one slant (c) was inoculated heavily

and another (d) with a dilution of the growth. About 10 cc. of broth were then added to the Kolle flask with a pipette, fair precautions being taken against contamination. All cultures were then incubated together. After 24 hours at 37°C. it was found that some sort of brown, slimy, unidentifiable material had grown in the flask, from which Staphylococcus aureus could be cultivated. Slant (a), inoculated from the plate with material containing staphylococcus colonies, contained staphylococci, while slant (b), inoculated with material from the same plate as slant (a), but away from the staphylococcus colonies, contained a pure culture of streptococci. Slants (c) and (d), inoculated from the Kolle flask, also contained pure cultures of streptococci. It would appear that the staphylococci which grew secondarily in the Kolle flask had been introduced there during the removal of material for the sucrose-agar slants, or with the broth added at that time.

It was now thought that, if large test-tube slants were used instead of plates or Kolle flasks, the possibility of contamination might be reduced. The first set of six tubes were inoculated as usual in the closed cabinet. One of these tubes showed the presence of Staphylococcus aureus and of plaques of bacteriophage. The author of the method in question does not mention having noted staphylococci with or without bacteriophage on the original blood-agar cultures, but does state that the sensitive type usually contains the bacteriophage.

Sensitive and resistant types of staphylococci were found in this culture. A tube of broth culture of the sensitive strain, after having been cleared by bacteriophage, was filtered through a Berkefeld filter which had been tested for defects just before use. The filtrate was found to be sterile by every test. Many workers have found that such supposedly sterile filtrates will, after being allowed to stand for a few days at room temperature, develop a secondary growth. Pryor describes an organism occurring in staphylococcus filtrates which was morphologically indistinguishable from B. diphtheriae. Our filtrate, obtained similarly but removed to sterile tubes, remained clear and sterile for over a month. (It was then discarded.) The above mentioned bacteriophage was the only specimen of lytic agent which we encountered.

Of the six blood-agar slants mentioned above, five were found to contain apparently pure cultures of streptococci and the growths were transferred to slants of sucrose-agar. The ensuing growths were found to be pure cultures of streptococci. By using proper precautions to avoid contamination we were able to repeat this result many times and to obtain 100 per cent pure cultures of streptococci on both blood-agar slants and on the sucrose-agar subcultures.

It is to be pointed out that the technique described by Pryor is ideal for picking up staphylococcus or other contaminations. In the blood-agar plates heavily inoculated with actively growing streptococci, chance contaminations by dormant staphylococci may be presumed to undergo a longer period of lag. The growth of the latter being more or less inhibited, they may remain invisible. When the entire growth is transferred to the special sucrose-agar, which is a medium rather poor for the growth of streptococci but very good for the growth of staphylococci, the latter organisms easily gain the ascendancy. It is also worthy of mention that in our work staphylococci were not the only types of contaminants, but that molds and gram-positive and gram-negative bacilli were also occasionally encountered.

The possible criticism that our results on the plates were due to needlessly careless technique, or that mutations have arbitrarily been regarded as contaminations, fails to explain why similar mutations did not occur on blood-agar slants in test-tubes. The relation between the type of vessel and technique used and the number of mutations or contaminations obtained is clearly shown in Table I. With the same technique we have obtained growths of staphylococci from cultures of streptococci from bovine mastitis, from impetigo, and with green-producing streptococci from feces. It was especially significant that when staphylococci appeared on the sucrose-agar slants, they could always be shown to have been on the original plates by reincubation of these.

We were not a little surprised by the frequency with which we obtained staphylococci or other foreign growths on the blood-agar plates treated as described above. These growths have not attracted our attention on blood-agar plates used for other work in the laboratory. It is to be noted, however, that for such work the operations of massive inoculation and later scraping of the entire surface of the medium

are not employed, only individual colonies being fished. In such work the appearance of one or more accidental surface contaminations is of no consequence and probably often passes unnoticed. In order to limit still further the possibility of accidental contamination, 32

TABLE I

Relationship between technique and the appearance of foreign growths

ORIGINAL CULTURES (BLOOD-AGAR) Ordinary technique (Petri plates)			SUBCULTURES (SUCROSE-AGAR) Showing growth of Staphylococci*
Inoculum	Plates made	Visible foreign growth	
Scarlet fever	26	12	11
Various	36	14	8
Sterile†	6	3	2
Totals	68	29	21
Special techniques (various)			
Inoculum and technique	Number of cultures	Visible foreign growth	
Kolle flask (scarlet fever)	1	0	0
Slants in test-tubes (scarlet fever)	24	1	1
Petri plates (scarlet fever)	15	1	5
Petri plates (sterile)†	14	2	11
Totals	54	4	17

Summary of Results of Original Cultures on Blood Agar

Percentage of foreign growths obtained with ordinary technique.....43
Percentage of foreign growths obtained with special technique..... 8

* In making massive transfers from plates, etc., showing visible contamination, an effort was made to avoid these until it was demonstrated to our satisfaction that with these precautions pure subcultures could frequently be obtained even from plates known to be contaminated. In this way a higher percentage of pure subcultures was obtained than when the whole growth was transferred to the sucrose-agar.

† These plates were treated with sterile broth. After incubation, whether they showed growth or not, scrapings from their entire surfaces were transferred to sucrose-agar slants.

blood-agar plates were poured with unusual precautions. Care was taken that the Petri dishes were thoroughly heated during sterilization. They were sterilized by dry heat in pasteboard cartons, a heat indicator being placed in the center of each carton. The inoculating cabinet

was wiped out with 5 per cent phenol solution, and the work table within the cabinet covered by a cloth wet with the antiseptic. The edges of the Petri dishes were flamed before they were opened. After pouring the blood-agar, the dishes were put back into the sterile cartons and incubated right side up. Three of the plates showed contaminating colonies on the next day and were discarded. The remaining 29 plates were taken into the cabinet which had been again wiped down with antiseptic as on the day before. The operator's hands and arms were scrubbed with the antiseptic as high as the elbows. The edges of the dishes were flamed. The entire surfaces of fifteen of the plates were inoculated with a broth culture of the scarlet fever streptococcus. The other fourteen plates were similarly smeared with sterile broth. The plates were again enclosed within the sterile cartons and incubated. After 24 hours one of the streptococcus plates and two of the control plates showed visible colonies of foreign growth. The cabinet was again prepared as before, the operator's hands and arms bathed with an antiseptic, the edges of the dishes flamed, and with a bent sterile platinum wire the surfaces of the plates scraped, the streptococcus plates and control plates alternately, and the growths transferred to sucrose-agar slants. The plates were again put into the cartons and reincubated. Five of the streptococcus plates and the corresponding sucrose-agar slants showed growth of foreign organisms, mostly staphylococci, and eleven of the control plates showed the growth of from a few to many thousands of contaminating colonies. When, at this juncture, only one or a few isolated colonies were present, it was assumed that the contamination had occurred at the time the plates were last opened and scraped. Where the colonies were thickly distributed over the entire medium, it was assumed that the contamination, though invisible, was already present on the medium at the time it was scraped, and that in this way the contamination was distributed over the entire plate. Naturally, the conditions on the control plates were more favorable for the growth of contaminations than on the plates heavily inoculated with streptococci, but it would seem quite as fair to assume that the growths on the control plates were the result of spontaneous generation as to assume that mutations had occurred on the streptococcus plates. (In Table I the above described plates are indicated by the legend "special technique".) It is apparent

that by this technique the number of contaminations or mutations was considerably reduced. Since foreign growths occurred but once on blood-agar slants in test-tubes, it is suspected that the plates became contaminated by currents of air drawn into the Petri dishes by the lifting of the covers.

From an inspection of the data described above it is readily seen that:

1. Where staphylococci occur on the sucrose-agar slants, whether they were visible on the blood-agar plates to begin with or not, they can always be shown to have been on the original plates by reincubation of these.

2. Staphylococci may appear on the reincubated plates without appearing on the sucrose-agar slants, indicating that the staphylococci were introduced onto the plates during or subsequently to the manipulations incident to making the massive transfer, unless it be assumed that they are mutations.

3. Where an assuredly pure culture is secured, and reincubation of the plate after removal of the streptococcus growth fails to bring out a growth of staphylococci, the growth on the sucrose-agar slant always remains pure.

4. The number of cases in which staphylococci (or other organisms) appear, either on the original plates or on the sucrose-agar slants, is in direct relation to the type of culture vessel and technique used.

5. It is possible to avoid the growth of staphylococci on both the original blood-agar and on the sucrose-agar subcultures by making the inoculations and transfers in a dust-free cabinet and by using large test-tubes or Kolle flasks instead of Petri dishes.

6. Growth of staphylococci will occur on a certain percentage of Petri dishes after the manipulations necessary for mass inoculation, mass transfer, etc., even when sterile broth and sterile instruments are employed.

CONCLUSIONS

In view of the abundant opportunities for contamination during the manipulations described by Pryor and others (3) working in the same field, the assumption that foreign growths are the results of mutation must be met with extreme conservatism.

REFERENCES

(1) D'HERELLE, F.: The Bacteriophage and Its Behavior. 1926. The Williams & Wilkins Company, Baltimore, Maryland.

(2) PRYOR, R. W.: The Cause of Scarlet Fever. *Amer. Jour. Public Health*, 1925, **15**, 847.

(3) For references to other work in the field of bacterial mutation see bibliography in d'Herelle's book referred to above; also in Löhnis', Life Cycles of Bacteria, Memoir 2, National Academy of Science, 1916. See also, HADLEY, P.: Microbic Dissociation. *Jour. Infect. Dis.*, 1927, **40**, 1.

BOOK REVIEWS

De Lamar Lectures, 1925–1926. The Johns Hopkins University, School of Hygiene and Public Health. 220 pp. $5.00. (*The Williams & Wilkins Company, Baltimore, 1927.*)

For the first time since their inception in 1920, the De Lamar Lectures have been published and made available to the general public. Thirteen lectures, each by a recognized authority, are included in the initial volume.

The range of the subjects is naturally very wide, since hygiene draws from every branch of the biological and medical sciences. Nevertheless, it is testimony to the liberal spirit prevailing in the School to see included a discussion of the prevention of automobile accidents by Mr. Robbins B. Stoeckel. While their prevention is primarily a police problem, increase in mortality from such accidents is causing serious concern, and study of the manner of their occurrence is well worthy of the attention of health authorities.

The present status of industrial toxicology in the United States is reviewed by Professor Alice Hamilton, and the varied requirements and difficulties of industrial housing are presented by Mr. Thomas Adams; Dr. Frederick W. Sears outlines the problems facing rural health officers.

The renewed interest in the ancient doctrine that certain types of humanity are predisposed to certain pathological states is evidenced by two papers; a statistical study by Dr. Dublin on body build in relation to longevity, and a critical discussion which distinguishes type from constitution in relation to disease, by Professor Stockard.

A pioneer in practical methods of prevention of simple goitre, Dr. David Marine outlines the present status of this subject; and Dr. Joseph Goldberger vigorously defends the theory of the dietary origin of pellagra. Dr. W. G. Smillie emphasizes the distinction originally made by Stiles, but not as yet well recognized, between disease and infection in hookworm. There are two papers on tuberculosis, one on methods of laboratory diagnosis by Dr. Edward R. Baldwin and the other on heliotherapy by Dr. Horace Lo Grasso.

In discussing the necessity for education in the control of cancer, Dr. Francis Carter Wood censures rather severely his professional brethren. His justification is that inexcusable mistakes are common and that in no disease of a chronic nature is delay in securing proper treatment so dangerous to the patient as in the cancerous group.

The series closes with a delightful essay on William Farr by Sir Arthur Newsholme.

The lectures were intended for a popular audience, and in editing for publication each author has obviously endeavored to make his presentation as non-technical

as possible. The volume may therefore be recommended to interested laymen as well as to health officers and physicians.

J. A. D.

Surgical Anatomy of the Human Body. by JOHN B. DEAVER. Second Edition. In three volumes. Thoroughly revised and rearranged. Volume I. Scalp, cranium, brain, face, mouth, throat, organs of special sense. 551 pp. $12.00. (*P. Blakiston's Son & Co., Philadelphia, Pa.*)

This volume is the first of a series of three representing the second edition of a publication which appeared twenty-five years ago. Advances in the surgical specialties during the last quarter century have been so great that many anatomical structures which formerly were of little import have now come in for more detailed consideration. It was with the purpose of keeping abreast of the modern knowledge that the second edition was found necessary. In this volume the chapter on the brain has been rewritten for the most part, and in this chapter, which is very well balanced as to subject matter, are found a host of anatomical structures connected in an admirable and interesting fashion with an abundance of clinical considerations. It is pointed out, for example, that some of the cranial nerves, like the facial, which were formerly considered to be purely motor in character, also have a sensory component. On the other hand, it is unfortunate to read a brief description of the lymphatics of the brain. The practical value of the book is again accentuated by the dissecting directions which are found throughout the text and which help to make the volume an entity. The illustrations are all good full-page plates, largely derived from the first edition and possessing therefore the curious segmented delineation of the arteries. The good, clear type, the excellent grade of paper, and the substantial binding bespeak the coöperation of the publisher. All in all, this book meets very well the requirements of an extended treatise on surgical anatomy.

F. C. L.

The Bacteriophage and Its Behavior. By F. D'HERELLE, M.D. Translated from the French by George H. Smith, Ph.D. 629 pp. $8.00. (*The Williams & Wilkins Company, Baltimore, 1926.*)

Probably the most interesting discussions of d'Herelle's phenomenon, from the viewpoint of the philosopher and biologist, center around the nature of the bacteriophage. In the book under discussion, d'Herelle presents very persuasive arguments and cites extremely ingenious experimental proofs in support of his own ideas. It seems to be fairly established that bacteriophagy is due to a live, autonomous, corpuscular being; an infra-visible, non-cellular protean micella which lives upon bacteria. It is said to have antigenic properties and that antisera can be prepared which inhibit its action.

The bacteriophage parasitizes bacteria in much the same manner that bacteria parasitize the higher plants and animals. It is said to enter the bacterial cell,

there to multiply and be visible, finally causing a rupture of the cell, with the liberation of a large number of new bacteriophage protobes.

The first part of the book is largely a detailed discussion of the factors influencing bacteriophagy, with descriptions of the phenomenon under various natural and experimental conditions. Methods are described and discussed.

An interesting analogy appears to exist between a bacterial population "infected" with a noxious bacteriophage and an animal population infected with a pathogenic bacterium. An epidemic occurs. Susceptible individuals succumb; resistant ones destroy the attacking agent, or they may become carriers. The bacteriophage may increase in virulence as well as in numbers. The bacteria, on the other hand, may respond by the acquisition of immunity. The analogy is practically complete.

To the bacteriologist and biologist, the most interesting sections of part one are those dealing with the formation of "ultrabacteria" and permanent mutations. Regarding the former, many workers have observed that after a broth culture of bacteria has been completely cleared by bacteriophage and then filtered through a Berkefeld filter, the filtrates, not infrequently but rather irregularly, show a secondary growth. These growths are attributed by d'Herelle to infra-visible forms of the lysed bacteria which pass the filter and then reassume the appearance of ordinary bacteria. The author and other investigators, whose work appears to be fully credited by d'Herelle, have found that these growths often have characters different from the original ones. They may differ culturally, morphologically and immunologically. They are, as a rule, no longer susceptible to the bacteriophage which caused their formation. In a word they are mutations. D'Herelle believes these forms of bacteria to be of common occurrence and that the viruses of rabies, variola, scarlet fever,[1] etc., are analogous to them and in some respects quite like them.

Regarding mutations, d'Herelle states that when bacteria acquire a resistance to a bacteriophage, they sometimes undergo very profound and permanent changes. His findings are confirmed and extended by other workers whose results d'Herelle accepts. To quote d'Herelle, "What actually happens is that through the influence of the resistance to the bacteriophage sudden mutations occur" (p. 219). These mutations may involve agglutinability, capsule formation, fermentation reactions, pigment formation, morphology and other characters, although not all characters are changed in every case. To use again the author's own words, "It is indeed probable that all of the fixed mutations occurring among bacterial species are produced through the action of the bacteriophage" (p. 222). "Biologists who have not kept in touch with the new facts disclosed by our knowledge of bacteriophagy may well be astonished at these statements, and they may even remain somewhat skeptical" (p. 225).

The clinical aspects of bacteriophagy are, finally, of greatest interest to the medical man. Briefly it may be said that d'Herelle regards resistance to diseases

[1] d'Herelle evidently believes scarlet fever to be due to a filterable virus.

like typhoid fever and to staphylococcus infections, as primarily, if not entirely, dependent upon the presence in the patient of a bacteriophage virulent for the infecting bacterium. The clinical course of the disease is paralleled closely by the daily variations in the virulence of the bacteriophage involved. ". . . . the acquisition of virulence by the intestinal bacteriophage for the pathogenic bacterium is the sine qua non for recovery" (p. 482).

Epidemics of such diseases among animals may be checked by the dissemination of fecal material of an animal which is resistant because of the presence, in its intestine, of a bacteriophage virulent for the bacterium causing the epidemic. "A diseased animal propagates his disease; an animal in a resistant condition propagates his immunity" (p. 503). Injections of bacteriophage suspensions are said to be extremely effective both prophylactically and therapeutically, but the immunizing agent in such suspensions seems to be in question. For one thing, suspensions of bacteriophage also contain the material of the lysed bacteria.

In the treatment of infected wounds, furunculosis, etc., small doses of appropriate bacteriophage suspension injected in or around the wound or applied on dressings are said to be effective.

It is pointed out that, since a given bacteriophage is seldom virulent for every strain of bacterium, only a known virulent and preferably an "autogenous" bacteriophage will prove effective in a given case. D'Herelle attributes the failure of many investigators along these lines to negligence on this point.

To sum up, it may be said that the book is a valuable contribution, and well worth reading. Each chapter is summarized very conveniently and the whole is well indexed. Nearly 650 references are cited. It is educational, stimulating and suggestive and full of the most astonishing statements. D'Herelle deplores the tendency of some authors to condemn or disregard his experiments and assertions without even taking the trouble to investigate his proofs. On the other hand, it is unfortunate that d'Herelle should commit himself so unreservedly to his own interpretation of certain phenomena which might be interpreted in several ways. One cannot, however, fail to be impressed with the evident sincerity of the writer who says, "If there are any who have not been able to isolate such[2] a race (of bacteriophage), I will gladly send them one upon request. These races will permit them to carry out all of the experiments described in this text." Thus the skeptic may believe through seeing.

M. F., Jr.

Transfusion of Blood. By Henry M. Feinblatt. 137 pp. $3.00. (*The Macmillan Company, New York, 1926.*)

This book, obviously designed for the practitioner, should be a considerable aid in popularizing the therapeutic value of blood transfusions. The dangers and untoward results are properly emphasized and clearly stated. Exposition, always

[2] D'Herelle claims that proper results can be obtained only with races of bacteriophage which are at maximum potency.

a difficult matter, is handled well and the various methods of transfusions are critically described in detail. The physiologic considerations are treated very briefly, but their extreme importance and relationship to recent modifications and advancements are adequately discussed. In general, the subject is interestingly presented and the material well arranged.

P. E. R.

Physiology and Biochemistry in Modern Medicine. By J. J. R. MACLEOD, assisted by R. G. PEARCE, A. C. REDFIELD, N. B. TAYLOR and J. M. D. OLMSTED. 5th Ed. xxxii + 1054 pp. 300 illustrations. $11.00. (*C. V. Mosby Company, St. Louis, 1926.*)

It is pleasing to note the evolution which this textbook has undergone since its first appearance only eight years ago. In the first edition the author dealt mainly with physiology and biochemistry in relation to the demands of routine medical practice. No place was given to many major branches of the subject, and students who were led to use the book found it disappointingly inadequate. Although several new chapters were incorporated in the third and fourth editions, many important functions of the neuromuscular system and the whole subject of the special senses were still left undiscussed. A decided step forward has been taken, however, in the compilation of the present volume. While retaining its original purpose, an attempt has been made, by further additions and the revision of every chapter, to present students with a survey of the science of physiology as it stands today. As a result of this expansion, an admirable treatise has now been produced which takes unquestionable rank among the first of its class.

The table of contents covering over one hundred chapters indicates the extent of the book. Most of the recent advances in the knowledge of the fundamental properties of neuromuscular tissues, as well as the older established facts, are introduced in a very readable section by Dr. Redfield. The physiology of the special senses—a new feature in this edition, by Dr. Olmsted—is well but briefly treated. Carbohydrate metabolism has been given excellent consideration, but it is regrettable that the question of urinary excretion should be so lightly passed over in only one short chapter. In revising the chapters on the ductless glands Dr. Taylor has given lucid treatment to a difficult subject. The references at the end of each section are adequate from the medical student's standpoint.

A perusal of the book evokes in the main a feeling of gratitude that Professor Macleod has performed a big task so thoroughly well.

S. W. B.

A Terminology of Disease. By ADRALIN V. S. LAMBERT, M.D. Third edition revised. 158 pp. $2.25. (*Paul B. Hoeber, Inc., New York, 1926.*)

This little volume, at present entering upon a third edition, provides a system of terminology of diseases and is designed to facilitate the classification of histories in hospitals. When one regards the bewildering array of names for diseases which are the possession of the modern physician, partly through inheritance and partly

through his own craving for originality, and considers that scarcely a disease can be mentioned which does not offer two or more synonymous terms for diagnosis, the absolute necessity of some sort of system is evident, if the data collected in any large hospital or dispensary are to be available for reference. When a choice has to be made between two terms as equally meritorious as trigeminal neuralgia and tic douloureux, as chicken-pox and varicella, as whooping cough and pertussis, it is likewise evident that such a system must often be arbitrary. In evolving his present method of classification Lambert has made use of two definite elements, the etiological factor and the anatomical structure involved. Thus, the volume opens with a list of conditions anatomically classified under a series of headings beginning with alimentary system, cardio-vascular system, etc., and is followed by an etiological classification which includes congenital malformations, disturbances of metabolism, diseases due to microörganisms, etc. The *specific form* in which a diagnosis is to be entered on a history is given in bold-faced type under the heading which indicates where a reference card should be filed. A considerable number of cross references have been included to facilitate finding the proper classification. Thus, in looking under the general anatomical heading of "Heart and Pericardium" for the proper term to be used in diagnosing a congenital malformation, one is referred to the heading "Congenital Malformations" appearing under the etiological classification.

The book closes with an index in which the names of the diagnostic terms are arranged alphabetically along with many synonyms, and the page reference for classification is given. This feature, the most important of the book, is notably incomplete. In the reviewer's opinion it makes little difference what sort of a filing system for disease conditions is adopted so long as the proper term and proper filing place are immediately accessible both to physicians and lay clerks. The only way this can be accomplished is by the use of a comprehensive alphabetical index containing all names for diseases in current use. As may frequently happen in a large hospital or dispensary, a physician not thoroughly familiar with the diagnostic system in use may be called upon to relieve temporarily the individual who regularly enters the diagnosis. The alphabetical index is the quickest way for him to locate the proper terms and often the only way consistent with the time at his disposal. Furthermore, when a mistake in form has occurred, it can be quickly righted by a clerk who has access to an alphabetical index. Thus terms such as coryza, ophthalmia neonatorum, Pott's disease, rubella, Brill's disease and even typhus fever, and many others which do not occur in the general index, should be included. The reviewer has found no titles in the book to cover such now well recognized disease entities as acrodynia, exanthema subitum, or nephrosis. He has also noted that separate headings appear for fragilitas ossium and osteogenesis imperfecta, terms which are synonymous.

A. A. W.

Diseases of the Skin. By RICHARD L. SUTTON, M.D., Professor of Diseases of the Skin, University of Kansas School of Medicine. Sixth Edition, revised and enlarged. 1303 pp. 1147 illustrations and 11 colored plates. $12.00. (*C. V. Mosby Company, St. Louis.*)

This book has been favorably reviewed in these columns on several previous occasions and there is little to say with reference to the present edition except to comment on the improvements that have been made. The author states that he has gotten rid of much of the old matter that has become obsolete, which is certainly a very commendable undertaking. The majority of our textbooks, at the present time, contain records of so much unsupported investigation as well as the personal preferences of various writers for therapeutic measures, that the physician or student is often lost when looking up facts essential in the diagnosis and treatment of cutaneous diseases.

A few of the more recently discovered dermatoses—the Fox-Fordyce disease, mycotic paronychia (Kingery-Thienes), congenital ectodermal aplasia, cheilitis actinica chronica (Ayres), ulcus vulvae acutum, glossitis rhombica mediana, achromia parasitica, and the dermatologic aspect of rat-bite fever—are included for the first time. There are also a number of new photographs and photomicrographs, as well as about 800 new references to the literature.

Sutton's book has become one of our most valuable textbooks on dermatology, and the author's untiring efforts in keeping it up to date makes it of special value to the dermatologist as well as to the general physician or student.

The book is very profusely illustrated with photographs most of which are good, but there are still a few which are quite under the standard of the majority of the illustrations.

L. W. K.

BOOKS RECEIVED

Report on Third International Congress of Military Medicine and Pharmacy. Paris, April, 1925. By COMMANDER WILLIAM SEAMAN BAINBRIDGE, M.C. 1926. 8°. 11 pages. Washington, D. C.

The Meaning of Disease. An Inquiry in the Field of Medical Philosophy. By WILLIAM A. WHITE, A.M., M.D. 1926. 8°. 220 pages. The Williams & Wilkins Company, Baltimore.

Clinical Application of Sunlight and Artificial Radiation. Including Their Physiological and Experimental Aspects with Special Reference to Tuberculosis. By EDGAR MAYER, M.D. 1926. 8°. 468 pages. The Williams & Wilkins Company, Baltimore. $10.00.

The Physiology of the Continuity of Life. By D. NOËL PATON, M.D., B.Sc., LL.D., F.R.S. 1926. 8°. 226 pages. Macmillan and Co., Ltd. London.

Pneumoconiosis [*Silicosis*]. A Roentgenological Study with Notes on Pathology. By HENRY K. PANCOAST, M.D., and EUGENE P. PENDERGRASS, M.D. 1926. 8°. 186 pages. Paul B. Hoeber, New York. $4.00.

Practical Surgery of the Joseph Price Hospital. By JAMES WILLIAM KENNEDY, M.D., F.A.C.S. Illustrated with 129 original half-tone plates, some in colors. 1926. 8°. 861 pages. F. A. Davis Company, Philadelphia. $10.00.

Shell Shock and Its Aftermath. By NORMAN FENTON, Ph.D., with an introduction by Thomas W. Salmon, M.D. Illustrated. 1926. 8°. 173 pages. The C. V. Mosby Company, St. Louis. $3.00.

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THE NERVOUS COMPLICATIONS OF VARIOLA, VACCINIA, AND VARICELLA WITH REPORT OF CASES[1]

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INTRODUCTION

For many years scattered instances of affections of the nervous system occurring in severe variola have appeared in medical literature, and similar complications even of varicella, although rare, have not been unknown, but textbooks contain no reference to any possible neurotropic properties of the virus of vaccinia. In the last few years mysterious cases of encephalomyelitis have been observed in Holland, Switzerland, and England, which seem to have a direct relation to vaccination. No instances of this nature have yet been reported in the United States. In view of the close biological relationship between variola and vaccinia and the clinical similarity between varicella and mild variola it is not astonishing to find that there is also a great similarity in the effect which these three infections produce on the nervous system. In another paper[2] one of the authors has shown that the nervous sequelae of many of the acute infectious diseases of childhood are due to the effect of the intoxication on the cardio-vascular system. In contrast to such conditions these three diseases probably produce a specific effect on the spinal cord and brain-stem. The anatomical picture is claimed to be characteristic and distinguishable from other inflammatory processes in the neuraxis. In the present paper it is proposed to review very briefly the literature and to append abstracts of case histories of patients who have been treated in the Harriet Lane Home for Children.

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THE NERVOUS COMPLICATIONS OF VARIOLA

The older medical writers have described a great variety of nervous complications of variola including delirium, psychoses, neuritides, both local and multiple, and even hemiplegias and aphasias. These conditions, of course, may occur as a result of any severe infection; they are not in any sense peculiar to variola, but are due to bacterial intoxication and invasion of the blood stream by secondary organisms. There is reason to believe, however, that certain neurological syndromes referable to the spinal cord and brain-stem are due to invasion of the nervous system by the virus of variola. This statement is supported by pathological as well as by clinical evidence. For convenience, the cases described in the literature may be placed in three groups.

The first and largest group contains cases in which paralysis of the legs occurred without other symptoms. Leroy d'Etiolles (1) described a case of this nature in 1856, and Gubler and Laborde (2), Spiller (3), and Damschino (4) have published similar instances. Patients described by Bernhart (5), Guttstadt (6), Westphal (7), Spiller, and Eichorst (8) had sphincter disturbances as well as paraplegia. In a few instances there was some loss of sensibility but most authors state that sensibility was unaffected. Flaccid weakness with muscular wasting and loss of tendon reflexes was the commonest picture, but in some cases the paralysis was spastic. In some case reports the plantar reflex is not mentioned so that polyneuritis is not altogether excluded. The clinical symptoms in general indicate an involvement of the anterior horn cells of the spinal cord. Death occurred in all but one of the cases cited, from bed-sores, urinary infection, or respiratory paralysis. The above mentioned authors give numerous references to case reports of a similar nature which have been omitted because the original papers are not available.

Bulbar symptoms with dysarthria and disturbances of swallowing have been noted by Gubler and Laborde, Guttstadt, Wohlrab (9), Combemale (10) and Aldrich (11). None of these patients died and substantial recovery supervened in most cases.

In a third group the signs of a more diffuse process in the nervous system were noted; including spastic weakness, tremor, ataxia, bulbar

disturbances, and in some cases mental changes. Westphal, Whipham and Meyers (12), Combemale, Spiller and Aldrich have described cases which fall into this group. In 12 out of 19 cases in which the date of onset was given, the nervous complications developed between the first and eighth days of the disease. In six instances the complications were noted before the eruption had appeared, while in four cases they were delayed until the second, third, and fourth weeks. Two of these four cases, however, were clinically atypical. It is evident that the early and fairly uniform appearance of nervous symptoms strongly supports the theory that they are due to the virus of variola and not to secondary infection.

Our knowledge of the pathological anatomy rests on 7 autopsies. The first post-mortem study was made by Damschino in 1871. Loss of cells in the anterior gray matter and scattered degeneration in the lateral columns with a reactive gliosis were found. There was a small softening in the lumbar region. Death had occurred six months after the illness. The same year Ponfick was unable to find any anatomical change in the nervous system in the case published by Guttstadt and again by Bernhardt. Westphal described the anatomical findings in two patients who had died several weeks after the onset. He found the lumbar cord to be most severely affected. Fat-laden phagocytes were found in masses in the gray matter and around all the vessels. Broad streaks of cellular infiltration spread out from the center into the white matter. Westphal could not be sure that there was much destruction of ganglion cells. Apparently no active inflammatory process was demonstrable and the areas of infiltration contained only phagocytes. The second case showed almost exactly similar lesions with the addition of a small softening in the thoracic cord. Spiller's two patients came to autopsy four and six weeks after the onset. In one no anatomical changes were demonstrable. In the other, there were severe degenerations in the anterior gray matter and scattered degenerations in the white matter of the middle thoracic region. There were focal and perivascular collections of phagocytes. Few ganglion cells were destroyed but changes in the Nissl bodies were demonstrable. In general, Spiller's observations were in agreement with those of Westphal. He says nothing of the brain. In all these cases the active process had apparently ceased before autopsy.

Eichorst was fortunate to secure the brain and spinal cord of a man who had died on the third day after his paralysis had occurred. No gross changes were to be seen. Microscopically, there was an inflammatory process in the whole spinal cord which was most pronounced in the lumbar enlargement and involved the gray matter more than the white. There were streaks of cellular infiltration extending out into the lateral and anterior columns. A few small hemorrhages were to be seen in the lumbar cord, but these were not extensive. The infiltrations consisted almost exclusively of mononuclear cells which were to be found both in the adventitia and in the nervous tissue outside of the adventitia. The meninges were involved in the lower part of the cord, but the spinal roots were not affected. The ganglion cells were surrounded by cells, but seemed to be preserved. No bacteria could be demonstrated. It is evident that essentially the same picture has been described by the various authors. All seven autopsies were performed in cases with spinal symptoms. In the cases with more elaborate symptoms it is probable that similar changes have developed in the brain-stem and basal ganglia. It is possible that the paraplegias in which no changes were found in the cord at autopsy were really instances of polyneuritis.

THE NERVOUS COMPLICATIONS OF VACCINIA

During 1925 and 1926 articles by Bastiaanse in Holland, Lucksch in Prague, Steiner, Blanc and Caminopetros in Switzerand, and an excellent article by Turnbull and McIntosh in England described over 50 cases in which the symptoms of a diffuse encephalitis or encephalomyelitis followed shortly after anti-small-pox vaccination. The present writers now add four additional cases to the list.

It seems clear the nervous complications are not due to any special strain of virus, because the cases have developed in widely separated areas where lymph from independent sources was employed. Children and young adults are, of course, most frequently affected. The local reaction has been severe in some cases, but in others of only average intensity. The onset is usually with headaches, vomiting, fever and drowsiness. These symptoms make their appearance about the tenth day on the average, varying between the extremes of four and fifteen days. The fever may reach 105°F. Retention of urine and con-

stipation are usual. General or focal convulsions sometimes occur. The pupils are unequal and there may be a squint. Stiffness of the neck and a positive Kernig's sign are not prominent but may be present. Trismus was considered a characteristic sign by Lusksch but was not found in Holland. The legs are weak or paralyzed and may be flaccid or somewhat rigid. The tendon reflexes are usually lost. Babinski's sign is frequently present. Bastiaanse thinks it is invariably so. Death occurs in nearly half of all cases, usually between the sixteenth and the eighteenth day. The acute stage lasts one or two weeks and it is unusual for any residual disability to remain. However, Turnbull and McIntosh refer to a case of Riddock's in which a partial paraplegia persisted, and in cases 3 and 4 of the authors' series some spasticity was still present after several months. All the continental patients who survived made complete recoveries. Complete cranial nerve palsies are not common and the most destructive lesions occur in the spinal cord. In two of the writers' cases the cerebral symptoms were overshadowed by the spinal damage so that the clinical picture was that of a disseminated myelitis. The spinal fluid findings are not especially characteristic. A moderate increase in white cells up to 60 or 70 may be found, or there may be no increase in cells. An increase in globulin is unusual.

The morbid anatomy has been briefly described by Lusksch as identical with that of epidemic encephalitis. Bastiaanse states that vaccinia involves the white matter more than epidemic encephalitis, but he did not examine the spinal cord. Turnbull and McIntosh have made a careful study of the lesions in 7 cases and describe a picture which they consider characteristic. The following description is based on their article. The gross changes are slight, merely some congestion of the meninges and a few red spots on the cut surface of the cord. Microscopically, in the acute stage, an inflammatory process is found throughout the whole central nervous system. The chief incidence is in the lumbar enlargement and the pons. The gray matter is more severely affected than the white, but both may show grave injury. All the smaller vessels are surrounded by collections of lymphocytes, plasma cells, leucocytes, and cells with elongated nuclei like fibroblasts. These cells lie in the adventitial sheath, but a peculiar feature of this condition is that there is also a marked cellular

accumulation in the nervous tissue outside the adventitia. The extra-adventitial infiltrations consist chiefly of free glial cells and leucocytes. Small circular areas of "softening and myelin destruction" are found about many of the vessels, often without any inflammatory infiltrations. Turnbull and McIntosh consider this a very characteristic feature of vaccinia encephalitis. The meninges show very little reaction. Hemorrhages are comparatively rare and small. Large softenings due to vascular occlusion are not found. Changes in the nerve cells are found in the form of tigrolysis, but complete necrosis is unusual. Turnbull and McIntosh could find no evidence of spread of the infection along the spinal nerves from the arm. The regional lymph nodes did not show any changes to suggest the entrance of an unusual virus. They make no mention of any attempt to demonstrate specific inclusion bodies in the nerve cells. Lucksch was unable to find such inclusions in the nervous system of his patients and also unable to demonstrate them in experimental vaccinia encephalitis.

Turnbull and McIntosh show that vaccinia encephalomyelitis differs anatomically from poliomyelitis in the absence of conspicuous areas of softening in the anterior gray matter, the rarity of nerve-cell necrosis, and in the presence of extra-adventitial infiltration with perivascular zones of myelin destruction. In vaccinia encephalomyelitis the lesions are most severe in the pons and lumbar cord; in epidemic encephalitis the maximal incidence is on the basal ganglia, while the lumbar cord is relatively spared in the majority of cases. The histological details in epidemic encephalitis are not very different from those of vaccinia encephalomyelitis, but again the perivascular infiltration is chiefly adventitial and the characteristic areas of myelin destruction are lacking. Turnbull and McIntosh believe that it is possible to distinguish vaccinia encephalomyelitis from all other non-suppurative inflammations of the nervous system.

Marie and Levaditi and his coworkers have produced encephalitis in rabbits by intra-cerebral injections of vaccine virus. Turnbull and McIntosh were able to produce a similar encephalitis by the injection of brain emulsions from their fatal cases, and also to demonstrate the presence of vaccine virus in this material by cutaneous inoculation. They found no evidence to suggest that they were dealing with a virus of exceptional neurotropic properties, and Lucksch

reached the same conclusions in his experiments with lymph from the same lot which had been used to vaccinate his patients. Bastiaanse (13) believes that the virus of epidemic encephalitis is latent in these children and is activated by the vaccination, so that the symptoms are due to both diseases. The evidence scarcely seems to support such a complex etiology.

Case 1. (Ped. 40377. Path. 8053.) *Girl of two years, eleven months, developed pains in the legs and drowsiness on the eighth day after vaccination. Stupor, trismus, opisthotonos, rigidity, clonic movements of the arms, increased tendon reflexes and extensor plantar responses were found on examination on the fourteenth day. Spinal fluid negative. Death. Diffuse encephalitis found at necropsy.*

E. G., white girl of two years and eleven months, developed pains in the legs and drowsiness eight days after vaccination. The next day the child stayed in bed and had some fever. On the thirteenth day there were two generalized convulsions and some stiffness of the jaws was noted. The child was then very ill and stuporous. On May 16, 1924, the fourteenth day after vaccination, the child was admitted to the hospital.

On examination the child was found to be well developed and well nourished. Pulse 120, respirations 32, temperature 102.6°F. She was stuporous but could be partially aroused. There were clonic movements of the arms at frequent intervals and a good deal of rigidity in all the extremities on passive movement. The head was retracted and the neck stiff. The jaws could not be fully opened. However, there was no rigidity of the abdomen or spasm of the diaphragm and no tonic spasms were caused by stimulation. The cranial nerves seemed normal. The pupils were small but equal. The fundi negative. The tendon reflexes were increased in the legs and there was a bilateral extensor response. The vaccination wound was healing and showed no sign of secondary infection. W.B.C. 38,200. Spinal fluid showed 6 cells, globulin 0, W.R. negative. Urine showed some albumin but was otherwise negative. Blood culture negative.

Course in hospital. In the next few days the rigidity of the jaws disappeared but the stupor deepened. Temperature ranged from 100° to 105°F. Large doses of tetanus antitoxin were given. No note was made about evidences of paralysis. On the eighteenth day general edema was noted and death occurred on the twenty-first day after vaccination. The clinical diagnosis was tetanus, although it was recognized that there were atypical features.

Pathological anatomy. The abdominal and thoracic viscera showed no pathological changes. Grossly, the brain and spinal cord were normal. Numerous sections were cut from the brain-stem, cortex and basal ganglia but only two from the spinal cord, both unfortunately in the thoracic region. A diffuse non-suppurative inflammatory process was seen in all parts of the central nervous system which was most severe in the pons and mid-brain and least severe in the cortex and thoracic cord. Perivascular cellular infiltrations were the most conspicuous

FIG. 1. SECTION FROM MIDBRAIN IN CASE 1, SHOWING CHARACTERISTIC ADVENTITIAL AND EXTRA-ADVENTITIAL CELLULAR INFILTRATION

lesions. These consisted for the most part of lymphocytes, but leucocytes, fat-laden phagocytes and the cells with elongated nuclei described by MacIntosh and Turnbull were also seen. Outside the adventitia there was usually a collection of amoeboid glial cells (see fig. 1). The perivascular areas which these authors described as softenings were also noted but were difficult to interpret. No hemorrhages or large softenings were to be found. No evidence of necrosis of the nerve cells could be made out. A few small areas of inflammatory reaction were found in the meninges, always in relation to a blood vessel. No Guarnieri's corpuscles could be found although a very careful search was made.

Case 2. (Ped. 15656. Path. 5310.) *Boy of six years complained of headache on the twelfth day after vaccination. On the fifteenth day there were squint, ptosis, weakness of the extremities, stiffness of the neck, a doubtful Kernig's sign, and bilateral extensor response. Spinal fluid negative. Death in convulsions.*

F. H., white boy of six years, was vaccinated and a severe local reaction developed. About twelve days later he developed headache, vomited and seemed feverish. The next morning there was a pain in the back and the child could not use his legs properly. Later in the day he seemed stuporous.

Examination on September 26, 1927, the fifteenth day after vaccination, showed a well developed and well nourished boy of six years in deep stupor. Temperature 101°F. There was a large inflamed area on the left arm at the site of vaccination. Head and eyes deviated to the right. Pupils sluggish but equal and circular. Optic fundi negative. Variable squint. Slight ptosis of the right eyelid. Right arm spastic; left arm and both legs flaccid. When disturbed, the patient moved the right arm in a jerky fashion, but did not move the left arm or either leg. The neck was slightly stiff and there was a suggestion of a positive Kernig's sign. The teeth could not be separated and the jaw muscles were rigid. Knee jerks difficult to obtain. The plantar reflex was extensor on both sides. Urine negative. Tuberculin skin test negative. Blood culture sterile. Widal test negative. Spinal fluid contained eight cells. Globulin test positive, Wassermann test negative; blood Wassermann test negative. Roentgenograms of the chest showed some root infiltration.

The spasm of the jaw muscles made nasal feeding necessary. The child grew progressively worse. The temperature rose to 108°, and the child died in convulsions sixteen days after vaccination.

At autopsy a few tuberculous nodules were found in the lungs, but the other viscera were normal. On section the brain was grossly normal. A few sections were cut to look for tubercles, but none were found and no diagnosis was made. When the case was reopened recently the brain could not be found, and only 6 sections were available: 3 from the cortex, one from the base, one from the spinal cord, and one included a part of the medulla at the level of the inferior olives. These sections were cut for the meninges and contained only small fragments of the nervous tissue. The spinal cord section showed no changes, but in all the

other 5 sections there were focal areas of perivascular cellular infiltration. There were striking collections of glial cells outside the vessel walls. Within the adventitia the cells were chiefly lymphocytes but polymorphonuclear leucocytes and phagocytes were also seen. As far as one could determine from the meager material the lesions were the same as those found in the first brain. No Guarnieri's corpuscles were found.

Case 3. (Ped. 33858.) *Girl of seven years grew drowsy and vomited a few days after vaccination. About three days later the child showed unequal pupils, spastic paralysis of the legs, retention of the urine and some sensory loss below the umbilicus. Spinal fluid contained forty cells. Almost complete recovery in two months. After four years there was slight mental defect some increase in tendon reflexes and marked obesity.*

G. M., white girl of seven years vomited one day and complained of pain in the back. The next day she was drowsy and ill and it was noticed that she could not move her legs. The child was brought to the hospital on the third day of the illness. No history could be obtained as the child was an orphan.

Examination October 6, 1922, the third day of the illness, showed a mentally alert normally developed child somewhat undernourished. Temperature 101.4°F. There was a large vaccination scar on the left arm which was partially healed. The right pupil was smaller than the left and there was some hyperemia of the retinae and optic nerve heads. The cranial nerves were otherwise quite normal. Both legs were completely paralysed and somewhat spastic. The knee and ankle jerks were much increased and a bilateral extensor response was obtained on plantar stimulation. The abdominal reflexes were not obtained. The spinal fluid contained forty mononuclear cells and the globulin test was positive. Gold Curve 432,110,000. Wassermann test in blood and spinal fluid negative. Roentgenograms of the spine negative. Tuberculin skin test was negative. W.B.C. 13,000. Urine contained some albumin but no pus. No definite statement was made about the jaw muscles, but it is clear that the mouth could be opened.

The next day the patient showed anesthesia to the umbilicus and there was retention of urine. Catheterization was necessary and a mild cystitis developed. On the ninth day the temperature fell to normal and there was some little movement in the toes. On the twelfth partial control of urine was regained and the legs could be moved in the bed. The sensory loss was now difficult to demonstrate. About two months after the onset the patient was discharged almost completely recovered. There was still a marked increase in the knee and ankle jerks and a questionable plantar response, but the child could control her urine and run about normally.

When seen again, March 3, 1926, the child was estimated to be retarded about three years mentally and had grown very obese. The skin was abnormally soft and delicate and there was excessive development of the breasts for a child of eleven. Pituitary disease was suspected and roentgenograms of the sella and

basal metabolism tests were made, but with negative results. The tendon reflexes were still increased in the legs and there was a bilateral patellar clonus.

Case 4. (Ped. 38556.) *Girl of six years developed fever and weakness of the legs with sphincter disturbance a few days after vaccination. Still spastic three months later.*

E. C., white girl of six years, was successfully vaccinated and shortly afterwards ill, vomited, was drowsy and feverish. A few days later it was noted that the child could not use her legs properly and there was some difficulty in urination.

Examination December 3, 1923, three months after the onset showed a poorly nourished child of six years. There was a severe paraplegia in flexion with increased tendon reflexes of the legs and a bilateral extensor response. Urination was difficult but there was not complete retention. No medical condition could be found. Urine negative. Tuberculin skin test negative. Roentgenogram of the spine negative. Unfortunately, the history in this case was incomplete and it was not possible to follow the child until a satisfactory examination could be made. It was subsequently heard that the child made a limited recovery.

In *summary* we may say that in our 4 cases of vaccinia encephalomyelitis death occurred in two instances and in the other two cases recovery was complete except for a slight residual spasticity of the legs. The onset of the nervous symptoms was on the eighth and twelfth days in two cases, but the exact dates could not be established in the other two. In two cases the cerebral symptoms overshadowed the spinal disturbances, but in the other two cases the clinical picture was that of a disseminated myelitis. In two cases trismus was a prominent feature and in one case this led to the mistaken diagnosis of tetanus. The spinal fluid was examined three times in the acute stage of the disease. The cell counts were 6, 8, and 40. In one case there was a positive globulin reaction, but the gold curve was always flat. An autopsy was secured in two instances. In one case histological examination showed a diffuse encephalitis corresponding in its details with the description of Turnbull and McIntosh. The other brain was not properly examined.

NERVOUS COMPLICATIONS OF VARICELLA

If we consider how commonly varicella occurs it becomes evident that involvement of the nervous system is extraordinarily rare. However, quite a number of instances of this nature have been observed, some of which seem to constitute a distinct group. If we

exclude the hemiplegias which Osler (20), Stirling (21), and Gordon (22), reported, the cases of "meningism" described by Koplik (23), and various doubtful cases, possibly of polyneuritis, which have been published by Rossi (24), Gay (25), Allaire (26) Camus and Sezary (27), and Stuchlik (28) as complications which are non-specific and might follow any severe infection, we find two groups of cases which apparently represent the effect of the varicella virus on the brain-stem and spinal cord, respectively.

In 1893 Marfan (29) described the case of a child of 22 months who developed ptosis of the eyelids and partial ophthalmoplegia a few days after the onset of chickenpox. Recovery was almost complete in a few months. A child observed by Menko (30) developed tremor and choreiform movements and an unsteady gait before the tenth day of chickenpox. Miller and Davidson (31) observed a similar case in which tremor developed on the fifth day with disturbances of speech and inability to stand. Caccia's (32) patient had tremor, vomiting and spastic weakness of the extremities more severe on the right side. In this case the nervous symptoms appeared on the fifth day of the disease. Rocaz and Lartigue (33) published the case of a child who exhibited choreiform movements of the left arm in the course of a mild case of chickenpox. Galli (34) has observed two cases in one family. The first patient developed vertigo, vomiting, unsteadiness of station and gait and a slight nystagmus on the fifth day of the eruption. This patient's sister showed identical but milder symptoms a few days later. The spinal fluid was negative in these cases. Winnicott and Gibbs (35) have recently published the history of a girl, 2 years and 10 months old, who developed nervous symptoms on the eleventh day of chickenpox. The symptoms included drowsiness, spastic weakness of the legs, urinary incontinence, increased tendon reflexes, bilateral extensor responses, difficulty in swallowing and articulation and variable squints. Spinal fluid negative. Recovery was complete in the course of a few weeks. Case 5 of our series evidently belongs in this same group. In a second very small group the symptoms are almost exclusively referable to the spinal cord. Wharton-Smith (36) described a case of spastic paraplegia in 1916. In this case and in case 7 of our series there were no cerebral symptoms demonstrable when the patient came under observation. In the case reported by

Krabbe (37) there were slight signs of brain-stem involvement in addition to a spastic paraplegia. The authors' three cases follow (case 6 was previously published by Wharton-Smith (36)):

Case 5. (Ped. 44620.) *Boy of nine years developed vertigo, vomiting and disturbances of gait on the seventh day of varicella. Nystagmus, choreiform movements of the arms and twitching of muscles soon appeared. Knee and ankle jerks increased. C.S.F. Cells 71, globulin 0. Prompt recovery except for chloreiform movements and a tendency to "sigh."*

J. W. L., white boy of nine years, developed vertigo and vomiting on the seventh day after the development of chickenpox. The next day he could not walk and was brought into the hospital. Three other children were healthy. The patient was considered healthy until two years before when during a routine examination he was found to have a positive skin tuberculin reaction. The mediastinal lymph nodes were found to be enlarged and there was some infiltration of the hilum of the lungs. He was sent to the state sanitarium for four months and then to a fresh air school. Until the chickenpox developed he had been apparently doing very well.

Examination. The patient was a well nourished boy of nine years who seemed rather drowsy and moderately ill. There were numerous vesicular lesions characteristic of chickenpox over the skin which were in the healing stage. The nose, ears, throat, chest and abdomen showed nothing abnormal on physical examination except for a possible slight enlargement of the spleen. The pupils were equal and circular and reacted promptly to light and during accommodation. The optic nerve heads and retinae showed a slight hyperemia, but nothing more. There was no diplopia, and ocular movements were full. On deviation to the left there was a coarse horizontal nystagmus with the slow phase to the right. The other cranial nerves were normal. There was no disturbance in hearing and no tinnitus. No definite weakness of the arms could be made out, but when the hands were outstretched, there were coarse irregular twitching movements of the fingers which were very similar to true choreic movements. Some fine twitching was to be seen in the left deltoid. Gait was very unsteady, possibly a little spastic, and the patient seemed to deviate to the right. Station was unsteady. The tendency was to fall to the right. No real weakness could be demonstrated in the legs. There was no sphincter disturbance. No sensory change could be demonstrated. The tendon reflexes were diminished in the arms and somewhat increased in the legs, but there was no clonus and the plantar responses were flexor. The Kernig's sign was negative and there was little or no stiffness of the neck. Blood W.R. negative; C.S.F. cells 71; mononuclear cells 93 per cent; polymorphs 7 per cent. The globulin test was negative; the mastic curve 0000000000000; Wassermann reaction negative. Urine negative. Red blood cells 5,150,000. White blood cells 6760; polymorphs 57 per cent; Hb. 98 per cent.

Course in hospital. In the next few days the child improved and seemed more

alert. The knee jerks became less active and the nystagmus and vertigo diminished. About six weeks after the onset the child showed the same choreiform movements of the arms and a slight general restlessness. There was no nystagmus or vertigo. The knee jerks were very active, perhaps more active than they had been when he was discharged several weeks before. Gait and station were normal. At times the patient would take a deep breath and expel it as if sighing. His mother said he did this frequently, but never before his recent illness. Nothing else was discovered in the examination.

Case 6. (Ped. 5385.) *Boy of seven years developed weakness of the legs and loss of sphincter control in the second week of varicella. Spastic weakness of the legs still present when seen two months later. Recovery incomplete at the end of a year.*

R. T., healthy white boy of seven years, developed a severe attack of chickenpox. About two weeks after the onset it was noticed that his legs were weak and that he could not use his left arm properly. A few days later retention of urine and incontinence of feces developed. The bladder had to be emptied by catheter. Both legs and the left arm were numb at this time and the patient could not feel a pin-prick in these areas. In the next few weeks the numbness and sphincter disturbance largely disappeared, but there was little improvement in the weakness in the legs.

Examination eight weeks after the onset showed a poorly nourished boy of seven years. The cranial nerves were normal. There was severe weakness of both legs which were strongly flexed at the hip and knee. Passive extension was difficult and painful. A small healing bed-sore was seen over the left buttock. No loss of sensibility could be made out. There was no incontinence. The knee jerks were obtained, but with some difficulty. The ankle jerks were increased and there was a bilateral ankle clonus. The plantar response was extensor on either side.

Laboratory examinations were as follows: W.B.C. 7200. Spinal fluid contained two cells; globulin 0; Wassermann test negative; gold curve 0000000000000. Roentgenogram of the spine negative. Blood Wassermann negative. Tuberculin skin test negative.

The patient was seen at frequent intervals for over a year. There was a gradual improvement in the strength of the legs. For about six months he walked with knees slightly bent, but when last seen walked normally. There was nocturnal incontinence every night, but this had been present before the illness. The tendon reflexes were still increased in the legs.

Case 7. (Ped. 35237.) *Boy of three years developed paralysis of the legs and loss of sphincter control on the seventh day of varicella. About two weeks later the patient showed only a mild spastic paraplegia. When last seen six weeks after onset was nearly well.*

D. T., negro boy of three years, developed varicella and about a week later, while the rash was still present, the patient grew drowsy and it was noticed that

he could not move his legs. After about four days some movement in the toes returned and improvement was rapid thereafter. Sphincter control was diminished but never entirely lost.

Examination two weeks after the onset showed a fairly well developed and nourished boy of three years. There was spastic weakness of both legs but no loss of sensibility. All the tendon reflexes were increased in the legs but the plantar response was flexor in type. Tuberculin skin test negative.

The patient was seen again six weeks after the onset when only a slight stiffness in the legs could be made out and some increase in the tendon reflexes.

We may sum up our observations as follows: Case 5 was that of a boy of nine years who was first seen on the eighth day of varicella, just 24 hours after the nervous symptoms had appeared. There were signs of a mild encephalomyelitis and the process seemed to be located chiefly in the brain-stem. Substantial recovery occurred in six weeks. In cases 6 and 7 the patients were first brought under observation eight and two weeks, respectively, after the onset and only spastic paraplegia was found on examination. In both cases recovery was almost complete.

In *summary* we may say that twelve cases of encephalomyelitis and myelitis complicating varicella have been published. The nervous symptoms have developed between the fourth and fifteenth days of the disease. The signs point to an involvement of the brain-stem and spinal cord and include ophthalmoplegias, nystagmus, vertigo, vomiting, tremor, choreiform movements, spastic weakness of the legs and sphincter disturbances. Sensory loss is unusual. In case 5, 71 mononuclear cells were found in the spinal fluid in the acute stage. There was no globulin, however, and the gold curve was flat. Recovery has been complete or nearly complete in every case. There have been no deaths and consequently the morbid anatomy is unknown.

CONCLUSIONS

It would seem, then, that a diffuse non-suppurative encephalomyelitis may occur as a rare but specific complication of variola, vaccinia and varicella. The clinical picture points chiefly to the spinal cord but signs of involvement of the brain-stem and even of the basal ganglia are found. In such cases the nervous symptoms develop after a fairly definite interval following the onset of the disease.

The vaccine virus has been demonstrated in the nervous tissues in cases of vaccinia encephalomyelitis, but this proof is still lacking in the encephalomyelitis which follows variola and varicella. The histological changes in vaccinia and variola encephalomyelitis are claimed by Turnbull and McIntosh to be characteristic and distinguishable from other types of non-suppurative inflammatory conditions of the nervous system such as epidemic encephalitis and poliomyelitis. The morbid anatomy of varicella encephalomyelitis is unknown. In all three conditions the tendency is for a more or less complete recovery. The relatively large number of cases of vaccinia encephalomyelitis which have been observed since attention has been directed to the subject suggests that the condition is less uncommon than has been believed. No doubt, many such cases have been mistaken for tetanus.

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FURTHER REMARKS CONCERNING TUMORS OF THE GLIOMA GROUP[1]

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INTRODUCTION

It is a matter of common experience in neurosurgical clinics that a diagnosis of glioma made on a surgical specimen gives no insight into the probable future clinical course of the patient from whom the specimen was removed. The need for more accurate understanding of these tumors led us to undertake to classify them in such a way that the diagnosis might have some significance for prognosis. We were very loath to undertake this task, and a cursory glance through the literature and at the pathological collection only increased our reluctance, for the literature seemed very vague and the structure of the lesions kaleidoscopic. Mallory (1) recognized only two types: *glioblastoma* and *neuroblastoma*. The latter tumor was acknowledged to be found only rarely in the brain. Ewing (2) may be taken as representative of the general literature. He distinguished three types: *astrocytoma*, *gliosarcoma* and *neuroepithelioma*. Under the last term he included the *neuroblastoma* of Wright (3). Masson's classification (4) was somewhat similar: *neuroépithéliome*, *gliome à petites cellules*, *gliomes à grandes cellules étoilées*. He added also *glioépendymome* and *glioganglíome*. Usually three major groups of gliomas were recognized with minor groups which varied with the different authors, but in every case it seemed that the description and definition of the different types were vague and incomplete, to say the least.

In the meantime, Dr. S. B. Wolbach (5) had been impressed by the frequency with which a certain type of glioma showed a tendency to form a stroma of connective tissue. These tumors he considered to be identical with the "neuroblastoma" of Wright. It is true that tumors

[1] Received for publication March 21, 1927.

of this type do contain frequently thin strands of connective tissue running between the numerous capillaries, but it was soon apparent that this was not constantly so, and the same formation might be seen in other gliomas of notably different structure.

Finally, in the autumn of 1922 a long and determined effort was begun to bring these tumors into some sort of order, and as guiding principles were adopted, (1) that new advances in scientific knowledge often follow the introduction of new technical methods; (2) that the proper investigation of the tumors or other pathological alterations of an organ should imply the use of the best known technical methods for displaying the normal structure of that organ; and (3) that one does not really know a thing until it can be expressed in mathematical terms. The first two considerations led to the discovery of a new method for staining neuroglial fibrillae (6) and, of far greater importance, to the discovery of the remarkable recent work of the Spanish school on the structure and development of the central nervous system (7).

It is unnecessary here to summarize their work. It has been recently and frequently done in many languages. Suffice it to note that del Río-Hortega in particular isolated and established the presence in the interstitial tissue of the brain of two cellular categories hitherto only vaguely suspected, which he called the *microglia* and the *oligodendroglia*. The embryological development of the classical neuroglia, or *macroglia* as it is now called, was studied especially by Castro (8). After a careful examination of their work and by using the technical procedures which they had elaborated, it was found possible to identify in tumors all of the various cellular types which they had distinguished in the developing and adult brain, with the exception of the microglia. Certain tumors could even be found composed predominantly of a single cellular type, notably in the case of the pineal parenchyma, the primitive spongioblast, the ependyma, the unipolar spongioblast, the astroblast, the astrocyte, the choroidal epithelium, and perhaps also the oligodendroglia and the apolar neuroblast.

It was decided to use these pure forms as much as possible, for to them a certain biological significance might be attached according to the embryological stage of development of the cellular type, and for the majority to group them according to the structure which most

frequently recurred. The result, after a long struggle, was the formation of 14 groups as follows:

1. Medullo-epithelioma.
2. Medulloblastoma.
3. Pineoblastoma.
4. Pinealoma.
5. Ependymoblastoma.
6. Ependymoma.
7. Neuro-epithelioma.
8. Spongioblastoma, (*a*) multiforme (*b*) unipolare.
9. Astroblastoma.
10. Astrocytoma, (*a*) protoplasmaticum (*b*) fibrillare.
11. Oligodendroglioma.
12. Neuroblastoma.
13. Ganglioneuroma.
14. Papilloma chorioideum.

A detailed description of these groups was given by Bailey and Cushing (9). The terminology adopted for them followed as far as possible accepted usage. For the rapidily growing gliomas usually known as *gliosarcoma* we adopted the term *spongioblastoma multiforme* after a conference with Globus and Strauss (10). This term is perhaps unfortunate, for the cells of these tumors reproduce only in the grossest way the structure of the spongioblasts of the developing nervous system, and we would prefer to call them *glioblastoma multiforme* as was our original intention. The following table will show the equivalent of the major groups of gliomas in the terminology of Bailey and Cushing as well as in the common pathological terminology.

In Table I the few tumors classified by Bailey and Cushing as medullo-epithelioma, pineoblastoma, and neuroblastoma, are neglected —eight tumors in all. We shall return to them later. The choroidal papillomas are not usually considered as gliomas. The remaining 10 major classifications have all been recognized in the literature, as we shall try to show in the following pages, and have a characteristic structure by which they may be identified. Between the different types there are all manner of transitions. Two types rarely found in the brain (neuro-epithelioma and ganglioneuroma) are admitted because of their frequent occurrence elsewhere in the nervous system.

TABLE I

		BAILEY AND CUSHING	GENERAL TERMINOLOGY
1	Medulloblastoma	Medulloblastoma Pineoblastoma	Neuroblastoma (Wright) Neurogliocytome embryonnaire (Masson) Glioma sarcomatodes (Borst, Fig. 105a)
2	Neuro-epithelioma	Neuro-epithelioma	Neuro-epithelioma (Flexner) Rétinocytome à stéphanocytes (Mawas) Blastoma ependymale (Marburg)
3	Spongioblastoma multiforme	Spongioblastoma multiforme	Spongioblastoma multiforme (Globus and Strauss) Gliosarcoma (Ewing, Borst) Gliome à petites cellules (Masson) Gliome polymorph (Roussy, Lhermitte and Cornil)
4	Pinealoma	Pinealoma	Pinealoma (Krabbe) Chorioma (Askanazy) Compound pineal-gland type (Skoog)
5	Spongioblastoma unipolare	Spongioblastoma unipolare	Neurinoma centrale (Josephy, MacPherson)
6	Astroblastoma	Astroblastoma	Neuroblastoma? (Greenfield)
7	Ependymoma	Ependymoma Ependymoblastoma	Ependymal glioma Glioépendymome (Masson)
8	Astrocytoma	Astrocytoma a. protoplasmic b. fibrillary	Astrocytoma (Ewing) Sternzellengliom (Stroebe)
9	Ganglioneuroma	Glanglioneuroma	Neuroglioma ganglionare (Bauman) Glioganglíome (Masson)
10	Oligodendroglioma(?)	Oligodendroglioma	Gliome à petites cellules rondes (Roussy, Lhermitte and Cornil)

CLASSIFICATION OF GLIOMAS OF THE BRAIN[2]

1. *Medulloblastoma.* There is frequently found in the brain, usually in the cerebellum of children, a tumor analogous in its structure to a certain cellular growth occurring elsewhere in the nervous system and known variously, according to its location, as *retinoblastoma* (*rétinocytome sans stéphanocytes* of Mawas), *sympathicoblastoma*, *meningoblastoma*, etc. One of the characteristic features of its structure is the presence of a fine fibrillary material consisting of delicate cytoplasmic processes of the cells, which cannot be stained sharply nor impregnated by any method. The nuclei have a tendency to group themselves so as to leave open spaces in which the fibrillary material is especially evident, the general structure resembling that of the medullary portion of the developing adrenal gland. These areas Wright called "rosettes," but it must be clearly understood that they are not analogous to the "rosettes" of the neuro-epithelioma of the retina, containing no lumen but only a tangle of fibrillary processes.

Wright considered this fibrillary material to be nerve fibres, and Mallory states that it reacts to stains as do nerve fibres. But when their argument is examined more closely, it is found to be based upon purely negative evidence. What they really intend to say is that this fibrillary material cannot be sharply stained by aniline blue nor by phosphotungstic acid-hematoxylin and, occurring as it does in the nervous system and being neither collagen nor neuroglia, it must consist of nerve fibres. This does not follow and, moreover, is probably incorrect. The fibrillary material has never been impregnated by specific methods for neurofibrillae. Herxheimer is supposed to have done so, but he pictures only rare and scattered fibrils whereas this material runs in broad bands. It is probable that, using Bielschowsky's method, he impregnated the reticulum of the connective tissue.

And this brings us to a second feature of these tumors, namely, the frequent overgrowth of reticular connective tissue, which was remarked by S. B. Wolbach (5) and independently of him by Masson

[2] A generic name for all the tumors originating from the medullary epithelium and from the neural crest is badly needed. Pick and Bielschowsky would doubtless put the 1st and 9th (perhaps also the 2nd) groups of this classification in their *System der Neurome*.

(11) and by Vanzetti. It may be completely absent and is in any case not pathognomonic of these tumors, but may be seen also in neuro-epitheliomas, in ependymomas and in pinealomas. What is usually seen is a great number of capillaries and running between them strands of reticulin resembling those seen in the cortex of general paralytics as first described by Ford Robertson. In certain instances these strands may be so numerous that the neoplastic cells lie in long rows between the reticular fibrils, which however have their own nuclei clearly distinguishable from those of the neoplastic cells. In other instances the neoplastic cells are subdivided into groups by the reticular stroma, giving to the growth an adenomatous appearance. In still other cases there is no stroma whatever.

The tendency of the nuclei to group themselves so as to leave open spaces filled with a delicate fibrillary material, and the numerous capillaries with their strands of reticulin, give to the tumor a characteristic appearance even with a hematoxylin and eosin stain under low magnification.

A third characteristic is the tendency of the neoplastic cells to differentiate into both nervous and neuroglial cells. Spongioblasts are often found, sometimes neuroblasts alone and more rarely both in the same tumor. Such a case has been recently described by Masson and Dreyfus (11) under the name of *neurogliocytome embryonnaire*. Their tumor was found in the vermis of the cerebellum of a child.

This double potentiality of the neoplastic cells seems to indicate that they represent an embryonic stage earlier than either the neuroblast or the spongioblast, corresponding probably to the indifferent cells of Schaper or, as they were called by Bailey and Cushing, *medulloblasts* (Neurogliocyt of Held and of Kohn).

The neoplastic cells themselves have very little cytoplasm around the nucleus. They are carrot-shaped, with one extremity streaming away in a long tail of delicate, lightly staining cytoplasm. The nucleus is oval, with a fine chromatinic network. Mitotic figures are numerous. With the hematoxylin and eosin stain the first indication of the differentiation of a neuroblast is a swelling and vesiculation of the nucleus (Fig. 1); of a spongioblast, the condensation of the cytoplasm so that the tail stains much more heavily with eosin. Later on, neurofibrillae may be demonstrated in the neuroblast and neuroglial fibrillae in the spongioblast, by proper impregnation methods.

Since the tumors of this type are still not generally recognized, a typical example which occurred in the cerebrum will be given in detail as an illustration.

Medulloblastoma of the right cerebral hemisphere. Three operations with removal of neoplastic tissue. Persistent radiotherapy.

History. On April 7, 1922, there was admitted to the P.B.B.H. (Surg. No. 16411) Frank T., a laborer, aged 37, referred by Dr. G. M. Crawford of Lorain, Ohio, complaining of headache, weakness of the left leg and convulsions. He had been well until 1919 when he began to have transitory attacks of tingling and numbness in the left hand. These attacks recurred every day, sometimes 2 or 3 times a day. In November, 1920, one of these sensory attacks ended in a convulsion during which he lost consciousness for an hour. He had since had three similar

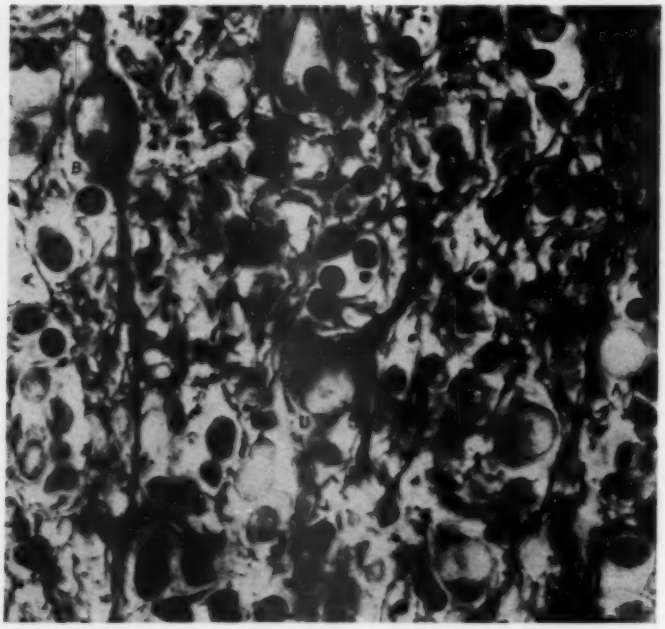

FIG. 1. Roger E., Surg. No. 24777. Differentiation of neuroblasts in a medulloblastoma. *A*, apolar neuroblast. *U*, unipolar neuroblast. *B*, bipolar neuroblast. Cajal's reduced silver method. × 850.

attacks, the last one followed by weakness of the left hand. In February, 1922, severe headaches appeared, usually in the occipital region. For a month he had been losing strength in his left leg.

Examination. There was found a left hemiparesis and hemihypaesthesia, predominating in the left arm, and bilateral choked discs of 2 diopters' elevation.

Course of Illness. On April 14, 1922, a right osteoplastic exploration was done, disclosing a soft protruding tumor measuring 5 x 5 cm. It was overlapping the brain and its margins could be dissected away with fairly clear demarcation,

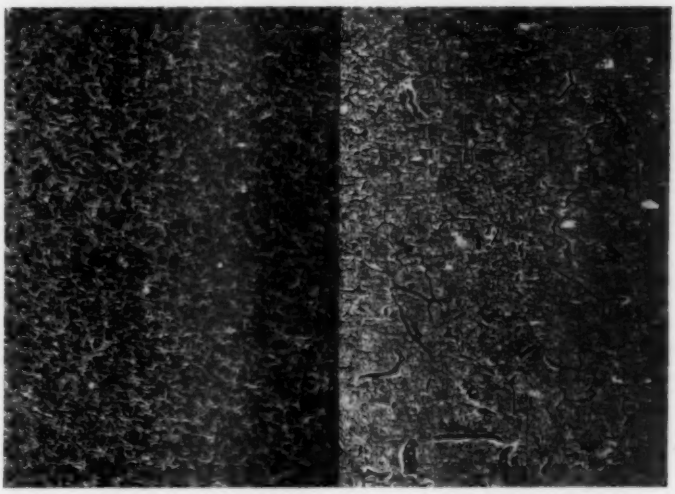

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FIG. 2. Frank T., Surg. No. 24753. Medulloblastoma. *a*. To show general structure. H. & E. × 80. *b*. Numerous capillaries with intercapillary reticular bridges. Perdrau's method. × 80. (Tissue fixed in Zenker's fluid, which accounts for the darkness of the background.)

though with a good deal of bleeding. The mass was finally tilted up from before backwards and a large cavity containing yellow fluid was opened. It was then possible, working from within outward, to remove the tumor apparently in its entirety. The patient recovered promptly and was discharged on May 3, 1922, with his condition practically unchanged, except that his headaches and choked discs were relieved.

Following his discharge he was given a series of 20 x-ray treatments over a period of 10 weeks. Later he was given treatments at intervals of about a month. In

August, 1922, he had an epileptic attack with loss of consciousness for half an hour.

Following this he had slight sensory attacks without loss of consciousness and felt quite well until December, 1924, when he was badly shaken up in an automobile accident. After that the attacks were more frequent in spite of bromide therapy, and the weakness of his left hand increased. In July, 1925, his headaches returned and constantly increased in severity. On September 9, 1925, he was readmitted (Surg. No. 24753).

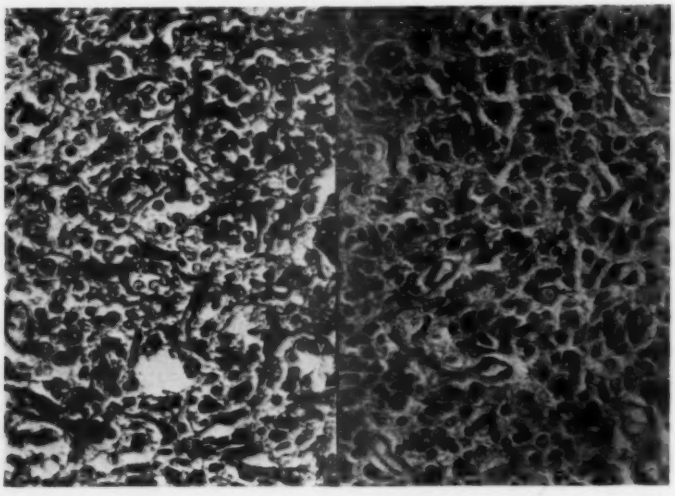

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FIG. 3. Frank T., Surg. No. 24753. Medulloblastoma. *a*. Several astroblasts are seen. Phosphotungstic acid-hematoxylin. × 300. *b*. One neuroblast is recognizable by its large vesicular nucleus. Methylene blue eosin. × 300.

Examination. There was found left hemihypaesthesia and hemiparesis predominating in the hand, and bilateral choked discs of 3 diopters' elevation.

Course of Illness. On September 22, 1925, the old boneflap was reflected disclosing a widely spreading glioma. Parts of the tumor were extremely soft and could be removed with the "sucker;" other parts were extremely tough so that they could be picked up and held with the forceps. There was also a cyst containing 30 cc. of yellow fluid which clotted on standing. A considerable portion of the tumor was removed. The patient recovered promptly from the operation and was discharged on October 9, 1925, with headaches and choked discs relieved but with a marked paresis of the left hand.

After discharge he received x-ray treatments every three weeks and remained free from headaches until February, 1926, when the weakness of the left arm and leg began to progress rapidly and severe headaches began. He became very irritable. He was readmitted on April 22, 1926 (Surg. No. 26202).

Examination. He was very dull, with tense and bulging decompression, bilateral choked discs and left spastic hemiparesis and hemihypaesthesia.

Course of Illness. On May 4, 1926, the old boneflap was re-elevated. An extensive soft tumor was disclosed, and a large mass 6 x 6 cm. was removed. The boneflap was also removed. He recovered slowly and was discharged on June 23,

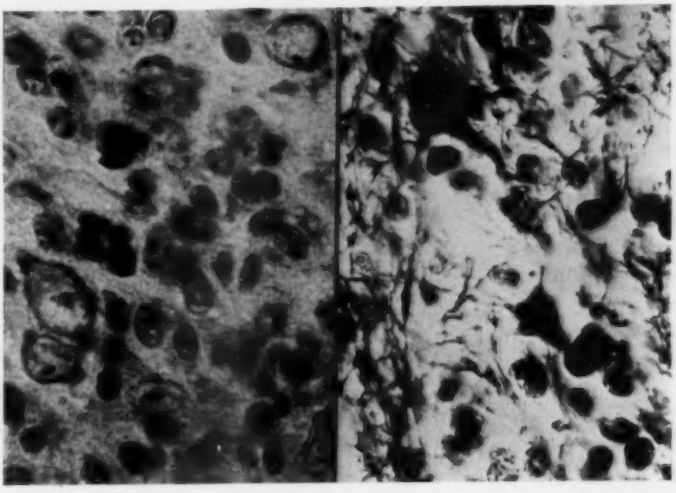

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FIG. 4. Frank T., Surg. No. 24753. Medulloblastoma. *a*. To show Nissl substance developing in neuroblasts. Bielschowsky-Plein. × 850. *b*. Neuroblasts with unmyelinated fibres. Cajal's reduced silver method. × 850.

1926, with his headaches relieved but with the decompressed area bulging badly and complete left hemiplegia.

Microscopical examination. The structure of the material removed at the three operations was essentially the same, but the tissue from the third operation was more necrotic, less differentiated and with many more mitotic figures. The illustrations are almost entirely from the tissue removed at the second operation, which was abundant and thoroughly examined. This tissue was fixed in 10 per cent formalin and in Zenker's fluid. The Zenker-fixed portion was embedded in paraffin and sections were stained with methylene blue eosin, phosphotungstic

acid-hematoxylin, neutral ethyl violet-orange G, and by the method of Bielschowsky-Plein. The formalin-fixed portion was cut in frozen sections which were stained with Hortega's IVth variant, Cajal's reduced silver, Cajal's gold sublimate, and by Perdrau's method.

The tumor is composed of small cells with a lightly stained scanty cytoplasm (Fig. 2a). The nuclei are oval with a delicate chromatinic network. There are numerous small capillaries, and between them may be seen strands of reticular

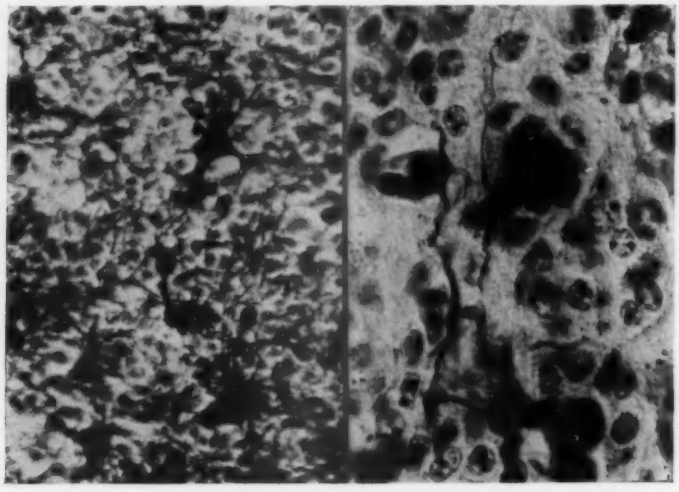

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FIG. 5. Frank T., Surg. No. 24753. Medulloblastoma. *a*. Astrocytes. Cajal's gold sublimate method. × 300. *b*. Neuroglial fibrillae differentiated in astrocyte. Neutral ethyl violet-orange G. × 850.

tissue (Fig. 2b). The nuclei have a tendency to group themselves around clear spaces filled by the delicate cytoplasmic processes of the cells. Occasionally a cell is seen with a larger, rounder, more vesicular nucleus having a heavy nucleolus and very little chromatin (Fig. 3b). These are neuroblasts, for in their cytoplasm may be identified Nissl bodies and neurofibrillæ (Fig. 4). The neurofibrillae found in the tumor could never be traced to any cell other than those having such large vesicular nuclei. In certain areas astroblasts may be found and even well-differentiated astrocytes with neuroglial fibrillæ (Figs. 3a and 5). Mitotic figures are numerous, especially in the material removed at the last operation.

The long survival of this patient, in view of the rapid growth of the tumor, must be ascribed to repeated operative removal and to the persistent radiotherapy.

Pineoblastoma. The cellular tumors of the pineal body, described by Horrax and Bailey as *pinealomas of spongioblastic type*, and called by Bailey and Cushing *pineoblastomas*, should probably be reckoned also in this group. They were originally separated because it was believed that the cells of the pineal *Anlage* had no potentiality of

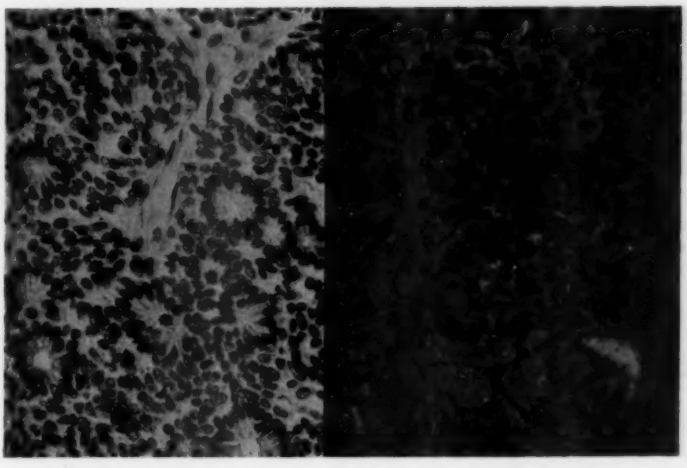

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FIG. 6. *a.* Neuro-epithelioma of the retina (*rétinocytome à stéphanocytes*). Copied from Mawas. *b.* Neuro-epithelioma of the cerebrum. Note the characteristic true rosettes. H. & E. × 300. John C., Surg. No. 15265.

developing into nervous cells. This opinion is now known to be incorrect, since we have observed in our clinic a typical ganglioneuroma of the pineal body. The cellular tumors of the pineal body should therefore probably be considered as analogous to the medulloblastomas and described simply as a sub-group of these tumors.

2. *Neuro-epithelioma.* The typical tumor of this group is the neuro-epithelioma of the retina (*rétinocytome à stéphanocytes* of Mawas). It is rarely found in other parts of the central nervous system. It has

a characteristic structure, containing small cavities around which cells radiate to form true *rosettes* (Fig. 6a). The cells of the rosettes contain at their inner ends blepharoplasten and sometimes cilia. The other extremity continues away into the tissue as a long cytoplasmic process which does not stain as do neuroglial fibrillae. The inner ends of the cells may project into the lumen of the rosette as do the rods

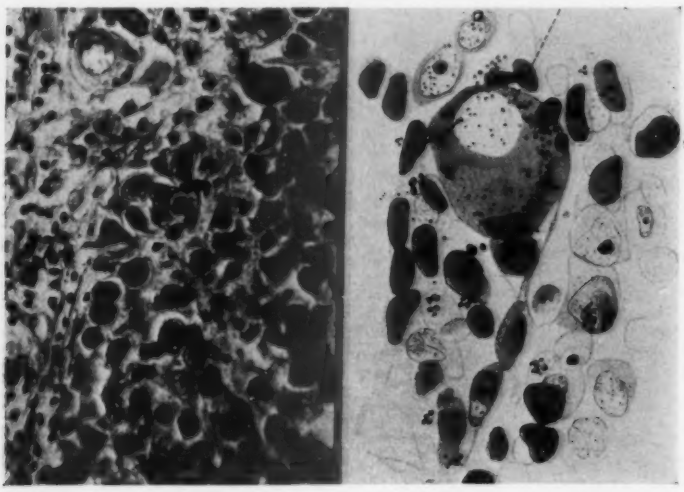

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FIG. 7. Neuroblastoma ? *a*. Hugh C., Surg. No. 24874. To the right, apolar neuroblasts (?). To the left, medulloblasts (?) *b*. Copied from Marchand. Called by him *Neurozytom*. Note the vesicular large nuclei.

and cones of the retina. The cells of the tissue outside the rosettes look much like those of a medulloblastoma (or retinoblastoma).

Such rosettes occur rarely in other gliomas, for example, the ependymomas (an instance will be given later) and occurred in abundance in the first of the cerebral tumors described by Bailey and Cushing as *neuroblastoma*. This tumor had previously been diagnosed by Dr. Wolbach as *neurocytoma*. However that may be, in certain areas were found numerous true rosettes having the same structure as those seen in neuro-epitheliomas of the retina. This tumor had probably better

be considered as a neuro-epithelioma (Fig. 6b). It contained mitotic figures but was of rather low malignancy. According to Mawas (13), the appearance of rosettes in retinal tumors is also an indication of lowered malignancy.

It seems to us now that perhaps too much weight was laid upon a positive result with Cajal's reduced silver in this case; the fact that the necropsy was done 20 hours after death leaving the specificity of the impregnation open to doubt. Moreover, the nuclei do not have the vesicular character seen in the neuroblast of the medulloblastomas. The other two tumors classed with it by Bailey and Cushing as neuroblastomas did not contain rosettes; one of them could, without doing violence to its structure in any way, be considered as a medulloblastoma in which an unusually extensive differentiation in the direction of neuroblasts had occurred, thus accounting for its long clinical course. The third tumor would remain in the unclassified group.

These considerations have led us to abandon the group of neuroblastomas as an entity, unless those described as *neuroblastoma apolare* be considered as such a group. Another similar tumor which seems to be composed mainly of apolar neuroblasts has recently occurred in the clinic (Fig. 7a), but unfortunately the material removed at operation was all fixed in Zenker's fluid so that the cells could not be positively identified as neuroblasts. Another tumor doubtless of the same nature was found by Marchand (13a) in the Gasserian ganglion. He called it a *Neurozytom* (Fig. 7b).

3. *Spongioblastoma multiforme.* This is one of the largest groups of gliomas. The tumors are rapidly-growing, anaplastic, dedifferentiating, and are essentially tumors of middle-age, occurring almost exclusively in the cerebral hemispheres. They are usually known as *gliosarcoma.* It was originally intended in this clinic to call them *glioblastoma multiforme.* Roussy, Lhermitte and Cornil (12) had already described such tumors as *gliomes polymorphes.* But Globus and Strauss had used the term *spongioblastoma* for them and we finally compromised on *spongioblastoma multiforme.* However, the cells of these tumors reproduce only in the grossest way the structure of the spongioblast of the developing nervous system. The terminology is being protested from various sources and seems unlikely to be generally adopted.

Histologically, their structure seems at first glance to be extremely varied, but we have already expressed in detail our belief that a large part of this variability is due to degenerative changes which are always extensive in these tumors. Nevertheless, spindle-celled, small round-celled, giant-celled (*Riesenzellengliom*, Otto Meyer) and other types may be distinguished, but there would seem to be little advantage in doing so. That the tumors are essentially similar is indicated by the age of onset and by the consistent rapidity of their evolution.

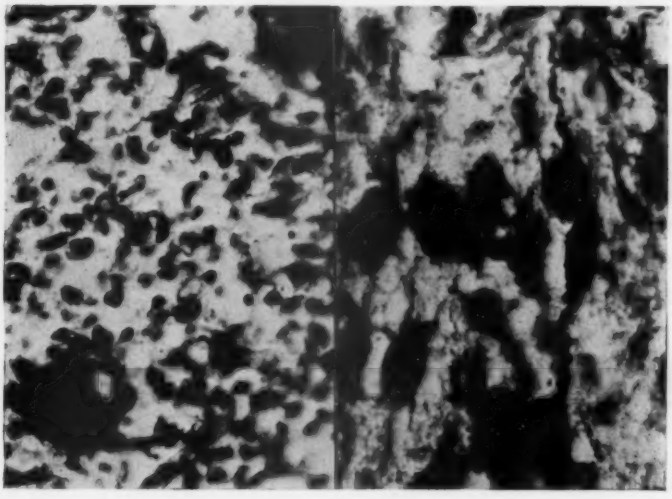

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FIG. 8. Spongioblastoma multiforme. Emory B., Surg. No. 23082. Cajal's gold sublimate method. *a*. × 300. *b*. × 850. Note the large bipolar spongioblast and small spider cell.

The cells have delicate, lightly staining cytoplasm, and large oval nuclei with a fine chromatinic network. The nuclei vary greatly in size and mitotic figures are numerous. True tumor giant cells are commonly seen. Where no degeneration has occurred, the neoplastic cells are usually bipolar but have a definite tendency to form astrocytes which in many cases gives to the ends of the bipolar cells a frayed-out appearance (Fig. 8). Fine neuroglial fibrillae are differentiated in their cytoplasm.

4. *Pinealoma.* The structure of these tumors is so characteristic that they can scarcely be mistaken. The neoplastic cells are large, globular, with abundant cytoplasm and a large, round nucleus. They are usually without processes, but occasionally have short stubby ones ending in bulbous terminations. In their cytoplasm may sometimes be seen groups of blepharoplasten. They are divided into large masses by an abundant reticulum in the meshes of which are numbers of small cells having the appearance of lymphocytes. Sometimes also neuroglial cells with fibrillae are found.

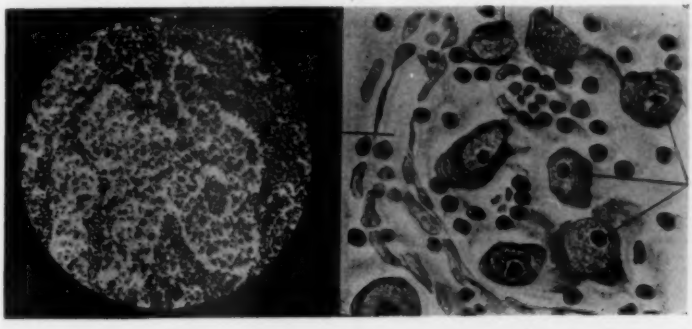

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FIG. 9. Pinealoma. *a*. Copied from Ley. Note the two types of tissue. *b*. Copied from Berblinger. To show the character of the parenchymal cells.

Many typical cases have recently appeared in the literature, among others those of Ley (14), of Berblinger (15) and of Kutscherenko (16) (Fig. 9).

5. *Spongioblastoma unipolare.* Closely allied tumors have been recently described in the literature under the name of *neurinoma centrale* by Josephy (17) (Fig. 10b) and by MacPherson (18) among others. The term seems inadvisable, for although there is a distinct resemblance in general architecture to the neurinomas of the acoustic nerve, still there is a most essential difference in microscopical structure. If a neurinoma of the acoustic nerve be impregnated by Perdrau's method there is revealed a most abundant formation of reticulin by the neoplastic cells (Fig. 10a). Such a formation is never seen in the tumors within the brain. The adjective *unipolare*

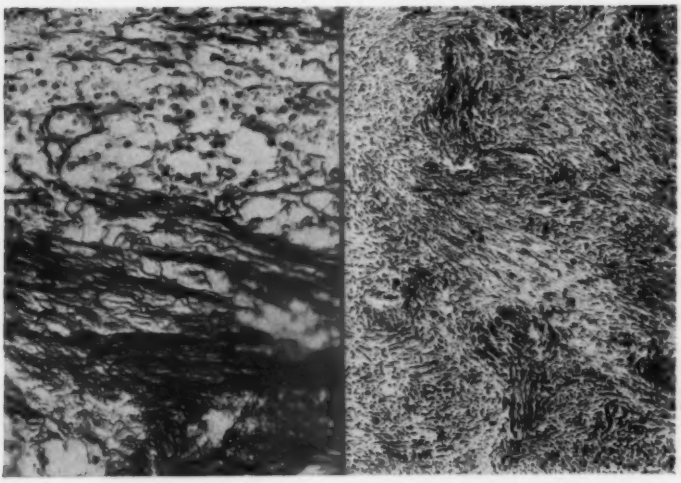

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FIG. 10. *a*. Eliza T., Surg. No. 26801. Neurinoma of the acoustic. Perdrau's method. × 300. Note the production of reticulin by the neoplastic cells. *b*. Spongioblastoma (neurinoma centrale). Copied from Josephy.

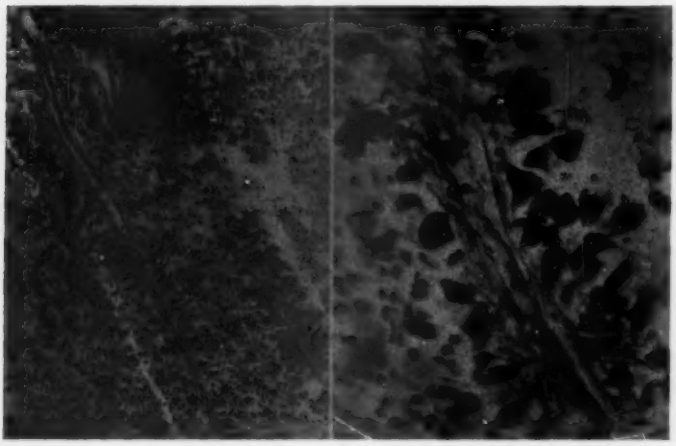

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FIG. 11. Astroblastoma. Copied from Greenfield. Note the stout perivascular feet of the neoplastic cells.

is inappropriate also since many of the cells are bipolar. It was added to distinguish this group from the spongioblastoma *multipolare*. If the term *glioblastoma* were adopted for the latter tumor, the confusion would not arise. It has seemed best at the present time, however, to retain the terminology as used by Bailey and Cushing.

The characteristic feature of the tumors in this group is formed by the processes of the cells. They may be unipolar or bipolar and may divide at some distance from the nucleus, but are in any case hard

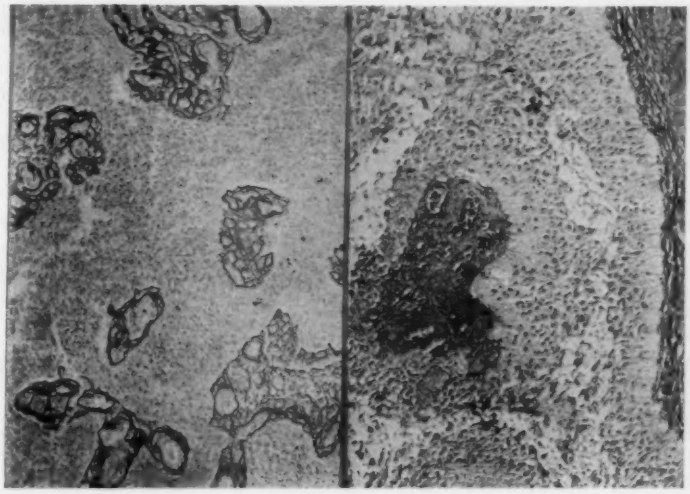

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FIG. 12. George P., Surg. No. 18864. Astroblastoma. To show general structure. *a*. Perdrau's method. × 80. *b*. H. & E. × 80.

and round like wires, staining intensely with eosin. No neuroglial fibrillae are differentiated, but the whole process stains feebly with phosphotungstic acid-hematoxylin or with ethyl violet-orange G. Mitotic figures are rare. These growths are essentially benign.

6. *Astroblastoma*. Astroblasts are often seen in gliomas, particularly in that form which we have called spongioblastoma multiforme and in the protoplasmic astrocytomas. Also, certain gliomas are almost exclusively composed of astroblasts. Greenfield (19) described three

such tumors under the name of *neuroblastoma* (?), because the cells were impregnated by the method of Bielschowsky (Fig. 11a). That these cells were in reality astroblasts is abundantly proven by the stout feet which stand on the vessel walls (Fig. 11b). The most characteristic tumor of this type which we have encountered is the one described clinically by Cushing in 1923 (Case 12) as a "non-fibrillar glioma of peculiar character" (Fig. 12). The astroblasts stand in long rows along the septa of connective tissue (Fig. 13a). There is

a b

FIG. 13. Astroblastomas. *a*. George P., Surg. No. 18864. To show row of perivascular feet. Phosphotungstic acid-hematoxylin. × 600. *b*. Gladys W., Surg. No. 26190. Showing more usual type of structure. Phosphotungstic acid-hematoxylin. × 300.

extensive degeneration between the vessels, as in Greenfield's case, and the centre of the tumor was occupied by a degenerative cyst. It is rare to see such septa of connective tissue. Usually the blood-vessels are scattered and the astroblasts radiate around them (Fig. 13b).

The neoplastic cells in these tumors are characterized by a somewhat triangular or pear-shaped body, from the smaller extremity of which extends a thick process, while at the opposite end are more delicate processes, so that the cell has a superficial resemblance in shape to the

pyramidal cells of the cortex. Such tumors have been described as ganglion-celled gliomas. The stout extension is not, however, an apical dendrite, but rather a sucker process which ends in a broad expansion on the wall of a blood-vessel and is stained heavily by phosphotungstic acid-hematoxylin.

7. *Ependymoma.* Attention has been called to the presence in the cells of these tumors of certain granules and short rods known as

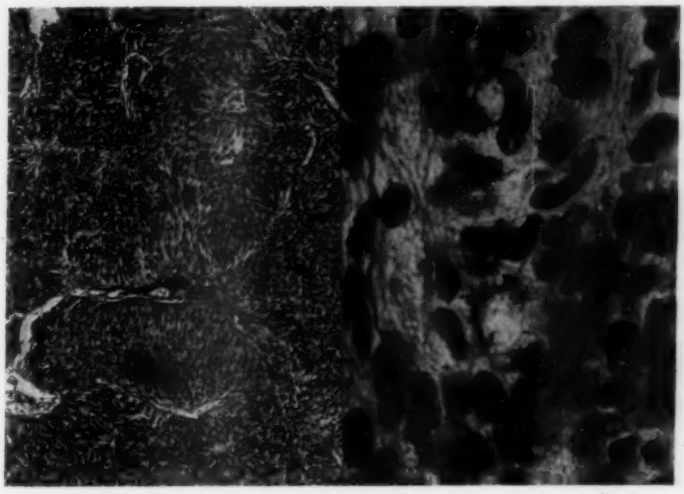

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FIG. 14. Ependymomas. *a*. Mary T., Surg. No. 26305. To show general structure. Hematoxylin-eosin. × 300. *b*. Lillian M. O., Surg. No. 24953. Showing rosettes similar to those of a neuro-epithelioma. Neutral ethyl violet-orange G (retouched). × 850.

blepharoplasten. The impression seems to have been given that the finding of these granules in the cells of a glioma is in itself sufficient to warrant a diagnosis of ependymoma. This is certainly incorrect and we have never maintained such an opinion. Our description has always begun by saying that these tumors are composed of polygonal cells with abundant heavy granular cytoplasm. Many of the cells, especially along the blood-vessels, have a long thick process which ends

on the vessel wall and stains very poorly with methods for neuroglial fibrillae (Fig. 14a). The cells differ from those of the astroblastomas in not having processes from the other extremity of the cells. The structure is also more compact and, *finally*, in the cytoplasm of the cells, usually in a clear area, occur groups of small granules and short rods resembling those seen in ependymal cells and there known as blepharoplasten.

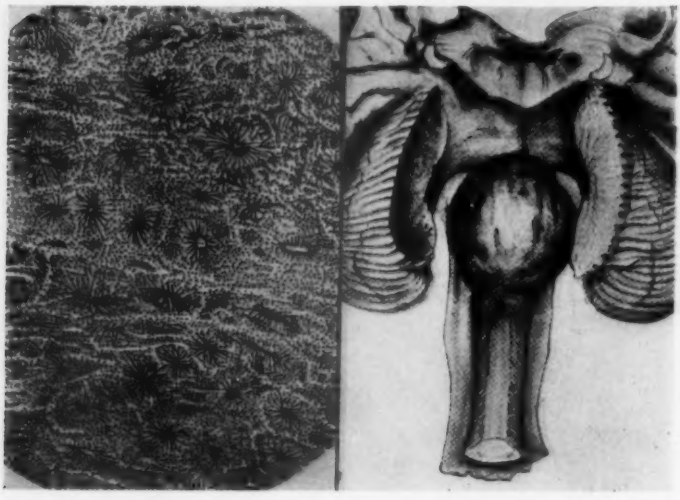

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FIG. 15. Ependymoma. Copied from Silberberg. *a*. Showing characteristic structure. The radiating cells centre upon blood-vessels and so do not form rosettes. *b*. Site of the tumor.

Some of these tumors in which a great number of processes were present have been described as *ependymoblastomas*. Occasionally there may also be found rosettes and larger cavities such as are characteristic of neuro-epitheliomas (Fig. 14b), differing only in that the blepharoplasten lie in a group in the cytoplasm instead of in a row along the free margin.

A typical tumor of this group, so far as one may judge from the description, was recently recorded by Silberberg (21) (Fig. 15), an

unusual papillomatous one by Orlandi (22) and two others possibly belonging to this class by Hirsch and Elliott (20). They occur near the cerebral ventricles, especially in the floor of the IVth ventricle.

8. *Astrocytoma.* These well-known lesions are composed of almost completely developed neuroglial cells, astrocytes, either fibrillary or protoplasmic, usually both (Fig. 16a). They are slowly growing tumors, the protoplasmic ones containing amitotic cellular divisions

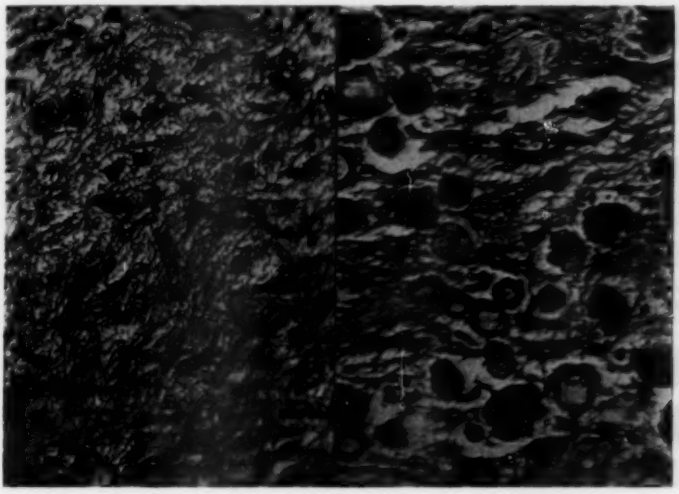

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FIG. 16. Astrocytomas *a*. Alice R., Surg. No. 26065. To show general structure. Phosphotungstic acid-hematoxylin. × 300. *b*. Ernest B., Surg. No. 25318. Neuroglial fibrillae and calcospherites. Phosphotungstic acid-hematoxylin. × 850.

and sometimes mitoses, the fibrillary ones rarely showing any evidence of cellular division. They are often calcified (Fig. 16b) and very often cystic. They occur both in the cerebrum and the cerebellum.

9. *Ganglioneuroma.* These tumors are rarely found in the central nervous system. In the head region they have occurred in the cerebellum, the medulla oblongata, the Gasserian ganglion, the tuber cinereum and in the sphenomaxillary fossa. Recently in this clinic

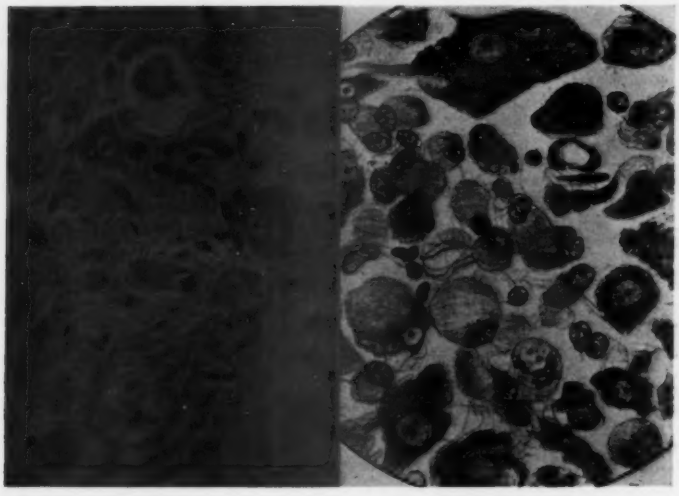

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FIG. 17. Ganglioneuromas. *a*. Edward R., Surg. No. 25210. Large nerve cells without Nissl bodies. Bielschowsky-Plein. × 300. *b*. Copied from Marinesco. Tumor in the infundibular region.

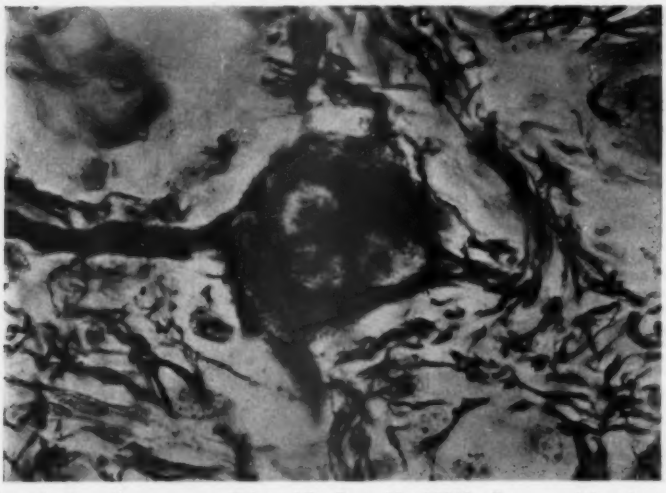

FIG. 18. Ganglioneuroma. Edward R., Surg. No. 25210. Large multinucleated nerve cell with neurofibrillae. Cajal's reduced silver method. × 850.

we have met with a typical case in the pineal body (Fig. 17a). These tumors are characterized by the presence of well-developed nervous cells with Nissl substance and neurofibrillae (Fig. 18), scattered among smaller cells analogous to those which form the neurinomas of the acoustic nerve. Occasionally astrocytes are also present. They are differentiated and relatively benign lesions.

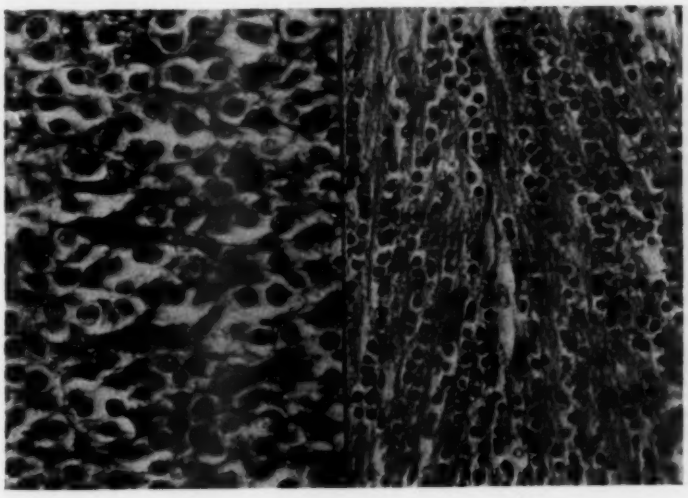

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FIG. 19. *a*. Oligodendroglioma? Delia K., Surg. No. 5490. Aniline blue-acid fuchsin. × 850. Protoplasmic astrocytes scattered among the oligodendroglial cells. *b*. Oligodendroglia (?). Cells differentiating in a medulloblastoma. David R., Surg. No. 23882. Phosphotungstic acid-hematoxylin. × 300. Note the spherical nuclei, heavily stained, and indefinite fibrillary material.

A typical case has recently been described by Marinesco (23). The tumor was situated in the infundibulum (Fig. 17b).

10. *Oligodendroglioma?* Finally, a tentative group of tumors must be separated which we have supposed to be formed of oligodendroglia, largely from the character of the nuclei of the neoplastic cells. These tumors are relatively benign lesions; cellular division is rarely seen. They are very prone to calcification. The nuclei are perfectly spheri-

cal for the most part, smaller than those of the neuroglial cells, with a heavy chromatinic network. There is usually by ordinary staining methods a clear halo around the nucleus, as around the normal oligodendroglia nuclei, and a delicate fibrillary substance which cannot be sharply stained, but is shown by Cajal's gold sublimate method to be formed by the processes of numerous scattered protoplasmic neuroglial cells (Fig. 19a). When this fibrillary material is slightly altered by degenerative change, it is stainable with aniline blue and gives to

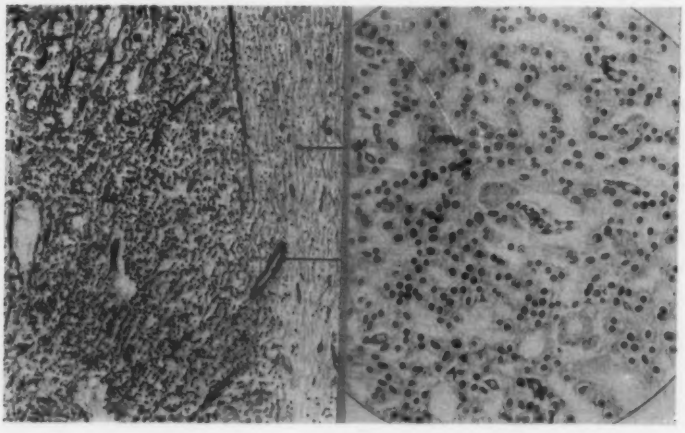

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FIG. 20. Oligodendroglioma (?). *a*. Copied from Roussy, Lhermitte and Cornil. Called by them *glioma à petites cellules*. *b*. Copied from Landau. Called by him "diffuse Gliom."

the microscopical preparation somewhat the appearance of the cross-section of a plant.

A tumor which must have been of this group was described by Roussy, Lhermitte and Cornil as a *"gliome à petites cellules rondes."* They believed it to be composed of microglia. We think it much more likely that the cells were of the nature of oligodendroglia (Fig. 20a). Another tumor certainly of this type has been reported by Laudau (24) (Fig. 20b).

Areas of such cells may be differentiated in other tumors. Recently

in a tumor from the central cerebellar region of a child such areas could be made out. Here were the usual small round nuclei with heavy chromatinic network and no mitoses. Between the nuclei were the the indefinite cytoplasmic strands (Fig. 19b). The remainder of the tumor was composed of larger cells with more vesicular nuclei in which mitoses were very frequent. Between these latter neoplastic cells was a most abundant stroma of reticular connective tissue, whose elongated heavily-stained nuclei could be easily distinguished. This tumor should probably be reckoned among the medulloblastomas, although neither neuroblasts nor spongioblasts could be recognized and the only differentiation of its cells seemed to be in the direction of the oligodendroglia.

The tumors of this group may be allied to the medulloblastomas. One of them after repeated operative interference now grows with the typical structure of a medulloblastoma containing numerous mitotic figures. In another recent one there have been found not only small astrocytes but even neuroblasts. Still this tumor was very benign, with a long clinical history. It may be that the medulloblasts occasionally differentiate into something allied to oligodendroglia and remain quiescent for long periods until stimulated into activity again by some unknown cause. It is not surprising that these tumors should be related to the medulloblastomas, for the oligodendroglia along the long fibre tracts of the brain develops from medulloblasts which wander into this region and there differentiate into either oligodendroglia or neuroglia.

The tumors showing this structure need considerably more study, especially with specific staining methods for the oligodendroglia, before their true nature can be established.

CRITICISM OF THE CLASSIFICATION

One of the first steps in the understanding of any group of natural phenomena is to make a classification. It has been said that gliomata are so varied in their structure that it is impossible to classify them. We have shown in the preceding section that there are many distinguishing characteristics around which numbers of them may be and have been grouped. It would perhaps be better to use the word "grouping" than "classification," for the principle adopted in group-

ing these tumors has been to choose certain types and around them to collect as many tumors as possible. Naturally, in practice one finds many pit-falls in the way of the actual classification of a given tumor, but this fact does not invalidate the attempt to isolate and describe types. A similar situation exists in the case of the tumors of the connective tissue, and in treatises on pathology one finds descriptions of osteoma, myxoma, chondroma, fibroma, etc., although in a specific instance one is obliged to diagnose osteo-chondro-myxo-fibrosarcoma. To take another example, types of nephritis are abstracted, although it is known that most cases are mixtures of these various typical lesions. Does not one still find in treatises on zoölogy differences of opinion as to the classification of certain animal forms?

In their monograph, Bailey and Cushing listed as *transitional* gliomas only those which seemed to be a mixture of two widely different groups. Many transitions occur between the protoplasmic and fibrillary gliomas, but they are so closely allied that the tumors were put in one or the other group according to the predominance of structure without separating any as transitional forms. The same is true of the ependymomas and ependymoblastomas. Of the five gliomas listed as transitional, two have been already noted as a sort of cross between a spongioblastoma multiforme and ependymoma ((24) Fig. 20; and (25) Fig. 28). Of the other three, one looks in part like a ganglioneuroma, with well developed ganglion cells and neuroglia. In these regions are no mitoses but many calcium deposits. In another part of the tumor there are many mitoses but no differentiated cells. The structure here looks like a medulloblastoma. Another is a very bizarre tumor resembling somewhat a spongioblastoma in certain areas, and in others more nearly an astrocytoma. There were many degenerative cysts. The remaining one was histologically a benign growth composed of spindle-shaped cells in which numerous neuroglial fibrillae were differentiated. There were no mitotic figures but many calcified plaques. Its general architecture was that of a spindle-celled spongioblastoma (Fig. 21), but in its indolent absence of growth, wealth of calcium deposit and fibrillary character, it resembled a fibrillary astrocytoma. We have since ceased to list gliomas as *transitional* and recognize only typical and atypical forms.

On the seventeen gliomas listed by Bailey and Cushing as *atypical*,

further study has succeeded in identifying two as medulloblastomas and one as an astroblastoma. Three others have already been described, (9) two as possible *neuroblastoma apolare* and one as a possible *microglioma*. Of the others it is interesting to notice three with long clinical histories (Minnie A., Surg. No. 5440; Cora G., Surg. No. 18039; and Julia K., Surg. No. 4581), all three patients having been submitted to repeated operations, radium and radiotherapy, etc. A

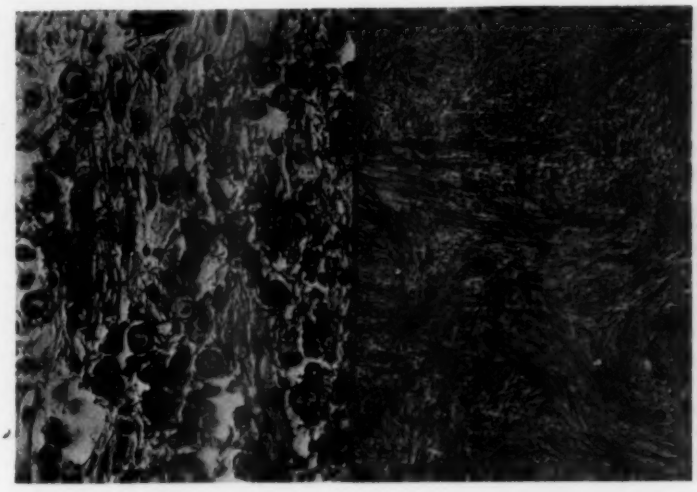

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FIG. 21. Transitional glioma. Jeanette W., Surg. No. 18229. General structure is that of a spindle-celled spongioblastoma; but the cells form abundant neuroglial fibrillae; no mitoses are present, and many calcified plaques are seen. These latter characteristics are common to the fibrillary astrocytomas. Phosphotungstic acid-hematoxylin. *a*. × 300. *b*. × 80.

postoperative infection and cerebral abscess also occurred in the case of Cora G., and the tumor removed shortly afterwards showed a very peculiar type of growth (Fig. 22). The final structure arrived at in each case at the last operation was very similar (Fig. 23), consisting of large rounded or polygonal cells with large nuclei and little or no fibrillary material. None of them was ever very typical of anything and all have remained unclassified.

Aside from the presence of atypical and transitional forms, another difficulty in classification exists in a surgical clinic such as the Brigham. Very often insufficient material is obtained, especially when necrosis has occurred in the tumor, to allow of a satisfactory examination, but we have been able to identify many such tumors at a second or even a third or fourth operation, and in many others, by necropsy secured by the coöperation of the parents or family physician. Nevertheless,

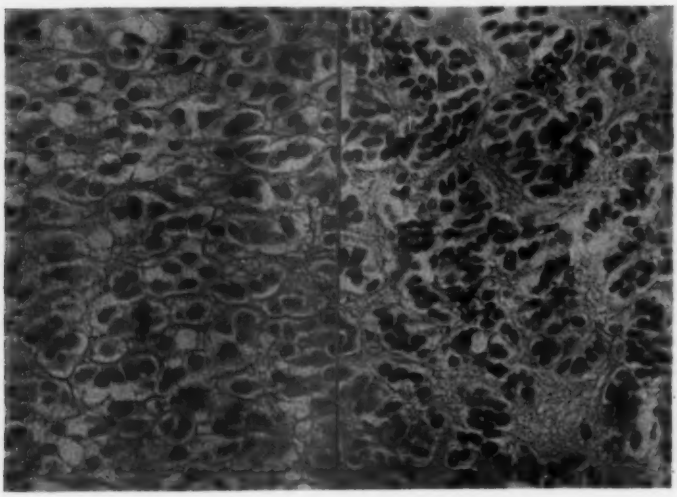

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FIG. 22. Cora G., Surg. No. 19109. To show the unusual type of growth adopted by a glioma which was infected. Methylene blue eosin. × 300.

a large number of surgical specimens must necessarily remain unclassified because the material obtained is insufficient or degenerated.

There is also the danger in working on surgical specimens that the particular portion removed may not be typical of the entire growth. This drawback may be largely minimized by taking care that there is no normal brain tissue included in the specimen—in which case one is probably dealing with the advancing edge of the tumor—and if one take care to estimate carefully how much of the architecture is due to degenerative change. For example, many tumors, and that recently,

are described as papillary, when the papillary appearance is clearly due to necrosis of the cells lying between the blood-vessels.

It has been urged also against the attempt to classify the gliomas in this way that the technique required is too difficult for general use. There is some justification for this criticism. An optimal examination of a glioma necessitates the use of three fixatives: 10 per cent neutral formalin, formalin-bromide and Zenker's fluid. But if one wish to

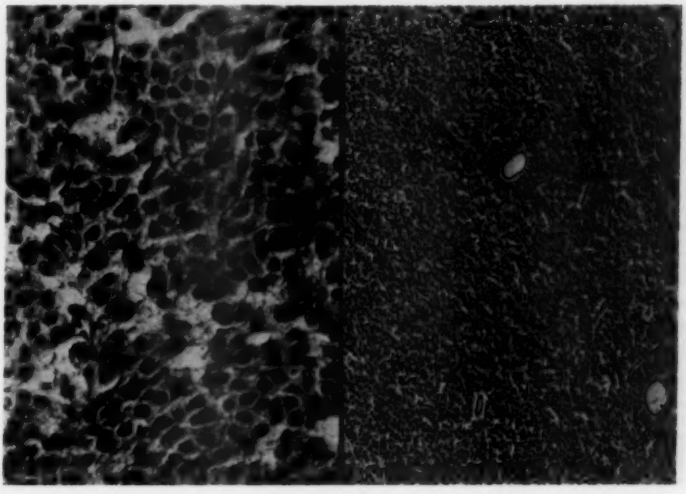

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FIG. 23. Julia K., Surg. No. 4581. To show the type of structure often adopted by gliomas when growing rapidly after repeated removals. Phosphotungstic acid-hematoxylin. *a*. × 300. *b*. × 80.

neglect the possibility of demonstrating a participation of the microglia and oligodendroglia in the formation of the tumor, a fairly satisfactory examination can be made by using formalin-fixed tissue alone. The various modifications of Hortega for Achúcarro's tannic-silver method would then be employed for the neuroglia. They are uncertain and not specific but often give most beautiful results. The other important structures are easily demonstrated on such formalin-fixed material.

It is much more instructive to work with frozen sections, for all the various procedures may then be carried out on consecutive sections from the same block and more nearly identical pictures obtained by the various methods. This is especially important because the examination should always begin with an estimate of the extent of degenerative change, and Herxheimer's fat stain demands frozen sections. The same sections may then be used for the demonstration of the neuroglia, neurofibrillae, nuclei, reticulin, collagen, elastin, myelin sheaths, etc., the Nissl substance alone perhaps necessitating paraffin or celloidin sections. It is easy enough, when the various procedures have been finished on frozen sections, to embed the remainder of the block in paraffin and continue its examination.

While these technical procedures were and will continue to be necessary for the positive identification of certain cells, still, after a little experience, one may identify the types of glioma simply from paraffin sections stained with hematoxylin and eosin, with a considerable degree of certainty. Neuroblasts, for example, have a typical nucleus, as we have seen, which is clearly shown by an ordinary nuclear stain. In textbooks of pathology one sees often represented sections of gliomas in which certain cells are labelled ganglion cells. That they are not ganglion cells may be stated with assurance from the character of their nuclei, in spite of the superficial resemblance. Perhaps the earliest sign of the differentiation of a neuroblast is the swelling and vesiculation of its nucleus. But the positive identification of such a cell demands other and collateral evidence, which can be furnished only by specific methods for Nissl substance, neurofibrillae, etc. In the examination of the tumors of the brain, therefore, as in the examination of the tumors of any organ of the body, one should not be satisfied with a routine nuclear stain but should employ the most specific methods known for demonstrating the normal structure of the organ.

It has been said also that since the overwhelming majority of gliomas fall into the three classes, medulloblastoma, spongioblastoma multiforme and astrocytoma, the other smaller groups are superfluous. Such an argument needs no answer. The surgeon is faced by a specific lesion, not by tumors in general, and so is the pathologist when he is called on for a diagnosis. The majority of tumors being sarcomas and carcinomas makes the study of teratomas none the less interesting and important.

While the gliomas in Dr. Cushing's collection were being grouped on a histological basis for the monograph of Bailey and Cushing, Miss Gerrard, Dr. Cushing's anaesthetist, was engaged in abstracting the clinical histories to determine the length of evolution, this being the clinical point the study was intended to elucidate and a convenient mathematical index of the significance of the classification. Her figures were carefully checked by Dr. Cushing. We were not unaware of the unsatisfactory nature of the material for such a statistical study and have made no attempt to minimize this defect, but the result was in a general way consistent with what one might expect from the character of the cells composing the various tumors, the more embryonic ones running a shorter clinical course. It was also consistent with the rapidity of growth of the tumors as judged by the number of mitotic figures. With the figures altered to agree with the classification herein adopted the result is as follows for the tumors up to January 1, 1924.

Average survival-period in months

1. Medulloblastoma	15
2. Neuroepithelioma	—
3. Spongioblastoma multiforme	12
4. Pinealoma	18
5. Ependymoma	25+
6. Astroblastoma	28+
7. Spongioblastoma unipolare	46+
8. Oligodendroglioma	66+
9. Ganglioneuroma	—
10. Astrocytoma	76+

This table brings out in the most striking manner that gliomas are not all hopeless affairs but that a large percentage of them are among the most favorable of intracranial neoplasms for surgical treatment, and these latter ones constitute no small percentage of the total.

Table II gives the number and distribution of the tumors in the various groups. They have been, since January 1, 1924, fairly consistent with the original conception of them given at that time by Bailey and Cushing. The only exception is for the spongioblastomas and oligodendrogliomas which have shown a somewhat low incidence. The clinical evolution of these groups in the last three years has also been fairly consistent with our original conception of them, although

we have no figures to submit, since too short a time has elapsed since January 1, 1927, for them to be significant.

There are several facts brought out by this table which seem worthy of mention, notably the much higher percentage of classified lesions. Up to January 1, 1924, only 51 per cent of the gliomas could by clas-

TABLE II

Gliomas—unclassified and classified

	TOTAL JANUARY 1, 1924		ADDITIONAL CASES IN 3-YEAR INTERVAL		TOTAL JANUARY 1, 1927	
A. Verified by cystic fluid alone		48		11		59
B. Excluded because differential study impossible		56		8		64
C. Unclassified gliomas of optic chiasm, pons, or midbrain		29		5		34
Optic chiasm	11		2		13	
Brain stem	18		3		21	
D. Excluded for other reasons		22		11		33
Atypical gliomas	17		11		28	
Transitional forms	5		0		5	
E. Classified		254		124		378
Neuro-epithelioma	1		0		1	
Medulloblastoma	35		20		55	
Pinealoma	5		3		8	
Ependymoma	12		4		16	
Spongioblastoma multiform	77		40		117	
Spongioblastoma unipolare	9		3		12	
Astroblastoma	14		6		20	
Astrocytoma	92		44		136	
Oligodendroglioma	9		3		12	
Ganglioneuroma	0		1		1	
		409		159		568
Double tumors		2				2
Net total		407				566

sified. From January 1, 1924, to January 1, 1927, nearly 80 per cent of the gliomas were classified. A great deal of this discrepancy is due to the fact that much of the older pathological material had become unsatisfactory for examination, but there is also another reason. A glance at the table will show the greatly lowered percentage of tumors verified by cystic fluid alone. An attempt is now made in every in-

stance to find and remove the mural nodule which exists in almost every cystic tumor of the brain. Nor is the surgeon at the present day satisfied with the removal of necrotic material, but searches for healthy appearing neoplastic tissue for examination.

The diminished number of verified tumors of the optic chiasm and brain-stem is doubtless the result of better surgical judgment which now leads to fewer operations for lesions of this sort.

The increasing interest and alertness of the general medical profession is reflected in the fact that in the last 3 years half as many gliomas have been verified in this clinic as in the previous 20 years.

We have no more than time to mention that this classification gives useful indications for Röntgen therapy. We have found it definitely valuable in retarding the growth of the medulloblastomas and spongioblastoma multiforme. In these cases we consider it advisable to push such treatment to the limit of tolerance.

As a guide to surgical treatment it is also most useful and with a recurrence of symptoms after operation we are much more encouraged to undertake a second intervention if the type of tumor removed at the previous operation was a benign one, the recurrence of symptoms having a greater chance to be due to refilling of a cyst, blockage of cerebrospinal fluid or other accessory factor.

Even though our main contention be not proven true, we feel encouraged, both Dr. Cushing and I, to continue the study of these interesting tumors in the belief that the time expended cannot be entirely in vain.

CONCLUSIONS

It seems possible to subdivide the majority of gliomas into groups each with a characteristic structure; to gain some insight into the reasons for their structural variability; and to attach to each group a certain prognostic significance.

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MORPHOLOGICAL STUDIES ON THE GALL-BLADDER[1]

I. A Note on the Development and the Microscopic Structure of the Normal Human Gall-Bladder

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For several years past the writer has been engaged in morphological and experimental studies concerning the orthology and pathology of the biliary passages with particular reference to the gall-bladder. In all, over a thousand post-mortem cases were examined more or less systematically to ascertain the gross structure and the anatomical and topographical relationships, as well as to secure data on anomalies of the gall-bladder and extrahepatic bile-ducts in man. For microscopic studies the biliary system of various animals, as well as that of human embryos, fetuses and new-born were used. These investigations were supplemented by studies of several hundred human gall-bladders removed by surgical operation. New approaches to a number of problems were made in several aspects of the general problem. In this communication—the first of a series setting forth the results of these investigations—a brief summary is given of our present knowledge of the microscopic anatomy of the normal human gall-bladder with some remarks on its development.

NOTES ON THE DEVELOPMENT

The gall-bladder, as well as the bile-ducts and the liver, are known to develop from a certain portion of the embryonic duodenum. From an embryological point of view they are therefore of endodermal and mesodermal origin. Endoderm gives rise to the epithelial lining of the gall-bladder and bile-ducts and to the parenchyma of the liver; mesoderm to the interstitium of the liver and to the rest of the layers of the bile-ducts and the gall-bladder.

[1] Received for publication April 13, 1927.

In Figure 1, a 25 mm. human embryo's gall-bladder is shown in cross section. The endodermal portion is represented by a single row of columnar epithelial cells lining the lumen; the rest of the wall is derived from mesoderm. A differentiation of this latter portion into layers is only slightly indicated, yet one may distinguish already a zona subepithelialis, a zona muscularis and a zona perimuscularis. The last is covered with peritonaeum on its free surface and is attached to the liver by the opposite surface.

The nuclei of the cells of the endodermal lining stand out very distinctly. The nuclei are large and contain deeply stained granules evenly distributed. The cytoplasm of these cells is stained lightly except at the very border of the free margin, where in the majority of the cells it stains somewhat more intensely. At this early stage of the development it is difficult to decide whether this narrow border which stains more deeply represents a cuticula. A few cells are seen in a state of mitotic division. In the mesodermal portion, immediately beneath the epithelium the cell nuclei are round and very numerous, whereas they gradually decrease in number and their shape changes from round to oblong as one passes towards the periphery of the gall-bladder. This change in the shape and the gradual decrease in number of the nuclei is at first alike on both the peritoneal and the liver surface of the gall-bladder. However, a sudden decrease in the number of the nuclei occurs half way towards the liver. Furthermore, there is a slight increase again where the viscus is attached to the liver as well as where it comes in contact with the peritonaeum.

The zona subepithelialis (1) is just beneath the epithelium: the cell nuclei are round here and very numerous. In the zona muscularis (2) from which the muscular coat is going to develop, the nuclei are less numerous and their shape is rather oblong. Young muscle cells and capillaries in process of formation may be recognized in this layer. Up to the periphery of this zone, the gall-bladder wall is alike on both its peritoneal and its liver surfaces. The next zone, the zona perimuscularis (3), is broader towards the liver, attaining a width almost equal to that of the zona subepithelialis and the zona muscularis. Cell nuclei are less numerous and they are evenly distributed. A few vessels also are seen in process of formation. On the periphery where this layer is attached to the liver an accumulation of round nuclei

may be observed. The relationship is somewhat different on the peritoneal aspect of the viscus where the perimuscular layer is merely a scarcely noticeable narrow zone between the muscularis and the peritoneal serosa.

In Figure 2, an 80 mm. human embryo's gall-bladder is shown in cross section. The differentiation into layers is more distinct. Also the formation of the folds of the mucosa has begun.

In Figure 3 the gall-bladder of a 130 mm. human fetus is shown in cross section. A further marked progress may be noted in the differentiation of the layers. The mucosa is developing its peculiar surface pattern.

Comparing for homologies the layers of the gall-bladder with those of the duodenum in different stages of their development, the question arises as to which of the two muscle layers of the duodenum is homologous to the muscularis of the gall-bladder; the circular and longitudinal

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FIG. 1. GALL-BLADDER OF A 25 MM. HUMAN EMBRYO IN CROSS SECTION. × 100

The single row of columnar epithelial cells lining the lumen is of endodermal origin; the rest of the wall is derived from mesoderm. The differentiation of this latter portion into layers has just begun, yet one may distinguish already (1) a zona subepithelialis (immediately beneath the epithelium where the cell nuclei are round and very numerous), (2) a zona muscularis (where the cell nuclei are less numerous and their shape is oblong; young muscle cells and capillaries in process of formation may be recognized in this layer), and (3) a zona perimuscularis (this is broader towards the liver than towards the peritoneal surface of the viscus where it is a narrow strip just between the muscularis and the covering serosa).

FIG. 2. GALL-BLADDER OF AN 80 MM. HUMAN EMBRYO IN CROSS SECTION. × 45

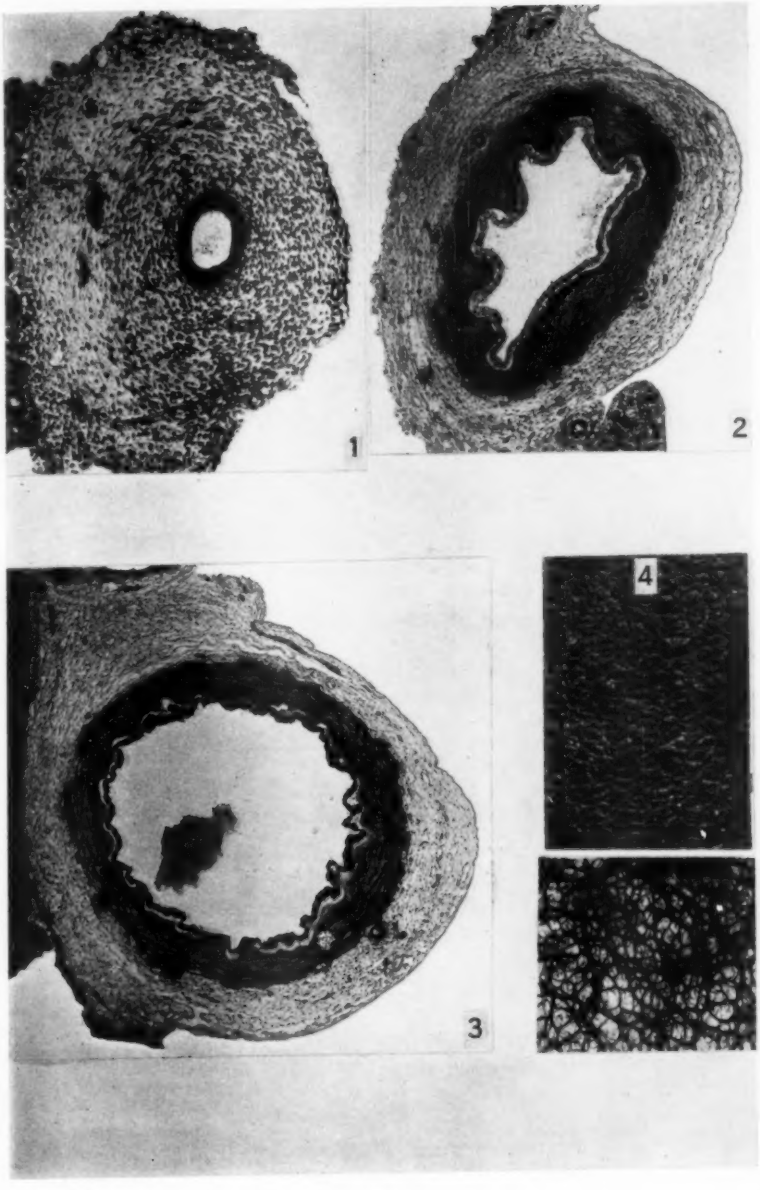
The differentiation into layers is more distinct than in the preceding case. The formation of folds of the muscosa has begun.

FIG. 3. GALL-BLADDER OF A 130 MM. HUMAN FETUS IN CROSS SECTION. × 30

A further marked progress is noted in the differentiation of the layers.

FIG. 4. INNER SURFACE PATTERN OF A NORMAL HUMAN GALL-BLADDER (ADULT)

The characteristic honeycomb-like appearance of the inner surface is recognized in the gross. Higher or primary folds enclose polygonal areas of about 4 to 12 square mm., each of which is subdivided by secondary folds into several smaller fields.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layers of the tunica muscularis or the muscularis mucosae? The closeness of the muscularis to the lining epithelium, the gall-bladder having no true submucosa, and its comparatively late appearance in development and also the fact that some of the glands occurring in the neck of the gall-bladder penetrate the muscularis just as Brunner's glands perforate the muscularis mucosae of the duodenum, suggest that genetically the muscular coat of the gall-bladder probably is a muscularis mucosae. This hypothesis would explain in a way why such direct mechanical, chemical or electrical stimuli, which cause an immediate and powerful contraction of the intestine, have no demonstrable effect on the gall-bladder.

MICROSCOPIC STRUCTURE OF THE NORMAL HUMAN GALL-BLADDER

The inner surface pattern. The inner surface of the gall-bladder has a honeycomb-like appearance, which is recognizable in the gross (Fig. 4). Details of the surface relief of the mucosa may be studied

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FIG. 5. PATTERN OF THE MUCOSA OF A NORMAL HUMAN GALL-BLADDER (ADULT), VIEWED BY TRANSILLUMINATION (XYLOL).

The primary and secondary folds anastomose freely with one another, hence the reticular appearance; the dark triple lines are blood vessels.

FIG. 6. GLANDS IN THE NECK OF AN ADULT HUMAN GALL-BLADDER REMOVED BY SURGICAL OPERATION (SCHMIEDEN'S CLINIC). × 29

In this cross section from the neck of a gall-bladder removed from a 24-year-old female patient, glands of tubulo-alveolar form are seen outside the muscularis. The gall-bladder contained two large and several small gall-stones. A slight cellular infiltration of the subepithelial layer and of the intermuscular septa was found, and no other changes.

FIG. 7. ROKITANSKY-ASCHOFF SINUSES IN AN ADULT HUMAN GALL-BLADDER REMOVED BY SURGICAL OPERATION (PROF. SCHMIEDEN): CROSS SECTION (ABOUT MIDDLE OF CORPUS). × 30

The mucosa is seen to dip down into the muscularis and protrude towards the external layers. These outpouchings of the mucosa which penetrate the muscular coat, termed here "Rokitansky-Aschoff sinuses" have often been confused with glands, although the two have no relation whatsoever to one another. This gall-bladder (removed from a male patient aged 52) contained a few larger stones about the size of cherries and a number of smaller facetted stones. Hypertrophy of the muscularis, marked cellular infiltration of the subepithelial layer, the intramuscular connective tissue and the perimuscular layer can be seen.

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best with a binocular microscope. The entire surface is seen neatly mapped out by higher or primary folds into polygonal areas of about 4 to 12 square millimeters, each of which is subdivided by secondary folds into several (3 to 9) smaller fields. The primary and secondary folds anastomose freely with one another, hence the reticular appearance shown in Figure 5. According to the height and arrangement of the surrounding folds, the depth, width and shape of the individual spaces vary considerably. Proceeding from the fundus towards the neck of the gall-bladder a more or less uniform decrease is noted in the size of the individual spaces and also a marked change in the height and arrangement of the folds of the mucosa. Towards the neck the primary folds assume a more longitudinal direction ending in the first fold of Heister which they help to form. Appearing again distal to the first Heisterian fold, they run somewhat parallel to the next one and end where this begins. While the primary folds disappear, the secondary folds richly cover both surfaces of the subsequent larger spiral-like Heisterian folds. In spite of the variations in the width and shape of the individual spaces and the existing differences just mentioned, in the human gall-bladder there is a characteristic arrangement of the folds in a surface pattern which is fairly constant. It must be said, however, that there are many factors, both intrinsic and extrinsic, which may change entirely the surface relief of the gall-bladder mucosa. As such, diseases of the gall-bladder itself (involving the mucosa only or also the other layers) have to be mentioned, but such extrinsic conditions which may lead to an overdistention of the viscus (e.g., the so-called functional biliary stasis, mechanical obstruction of the choledochus, etc.) have also to be considered.

The mucosa. In a histological section (Figs. 6 to 12) the inner surface of the gall-bladder is seen to be lined by a single row of uniform columnar epithelial cells resting upon a scant connective-tissue layer, the lamina propria mucosae, or subepithelium. The gall-bladder has no submucosa. Near the basal end of each epithelial cell, a deeply stained evenly granulated nucleus is seen within a much lighter stained cytoplasm which shows no cuticula on the free margin. The contours of the cells are usually very distinct. No specific cells of secretion, goblet cells or glands are seen to interrupt the uniformity of the cells of the lining epithelium. An exception to this rule is found

in the neck of the gall-bladder where glands of alveolar and tubulo-alveolar form are occasionally seen (Fig. 6). Deeper outpouchings of the gall-bladder mucosa towards the external layers, i.e., Rokitansky-Aschoff sinuses (Fig. 7), have often been confused with these glands (literature by Shikinami), although the two have no relation whatsoever to one another. Usually the glands may easily be recognized by the staining reaction and size of their cells, and by the shape and position of their cell nuclei. The cells of the glands are large and very lightly stained; their nuclei are small and flat, and appear crowded to the basal ends of the cells.

The lamina propria mucosae or subepithelium, consisting of the connective-tissue layer between the epithelial lining and the muscularis, is usually very thin, sometimes barely sufficient in extent to accommodate the rich vascular net of the mucosa (plexus subepithelialis). The gall-bladder, as said before, has no submucosa. Sometimes there are cells in the subepithelial layer (Mallory calls them endothelial leucocytes) loaded with anisotropic lipoid substances. The folds of the mucosa appear in such cases much larger and plumper than usual (Figs. 8, 9). Epithelial cells of the gall-bladder mucosa containing lipoid substances were first seen by Virchow (1846). He regarded these cells later (1857) as being concerned in the intermediate metabolism of fats, considering a part of the fats of the bile to be reabsorbed by the gall-bladder mucosa. Thus Virchow concluded 70 years ago that "the gall-bladder is not a mere reservoir but has a further function, for through it fats find an intermediate passage way back into the circulation." According to Testut, Naunyn and others, lipoids are produced and not resorbed by the gall-bladder mucosa. Newer experimental and morphological observations by Aschoff and Küttner, Policard, Boyd, Kusnetzowsky, Sweet, Halpert, Mentzer and others, support the view originated by Virchow that lipoid substances are taken up from the contents of the gall-bladder by the cells of the epithelium and thereby passed to the underlying cells.

According to Aschoff and Bacmeister the lamina propria mucosae may be divided into two layers; a more cellular "subepithelial layer" which contributes primarily to the formation of the folds, and the "mucosal layer proper" which is rich in fibers and is somewhat less cellular. Considering, however, the usual thinness of the connective-

tissue layer between the epithelium and the muscularis such a differentiation seems rather artificial.

The *muscularis* is a very thin coat consisting of longitudinal, oblique, and circular muscle fibers in delicate bundles none of which is, however, dense enough to form a distinct and separate layer. Some information may be gained as to their arrangement by comparing longitudinal and transverse sections taken from the same gall-bladder. In a transverse section of a somewhat contracted gall-bladder, muscle fibers, for the most part gathered into longitudinal bundles, are seen in cross section next to the mucosa. Outside of these are found more or less longitudinal sections of some oblique or circular muscle bundles (Figs. 7, 11). In a longitudinal section, of course, conditions are just the reverse. The inner bundles, next to the mucosa, are mostly longitudinal sections of longitudinal bundles and the outer ones, next to the perimuscularis, are cross sections of circular or oblique muscle

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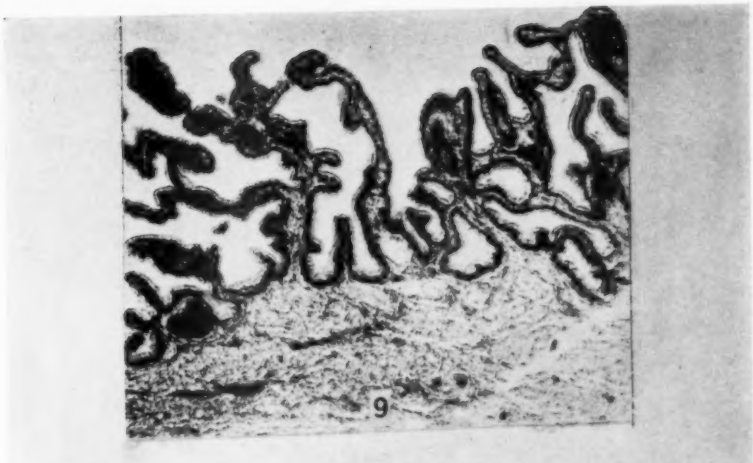
FIGS. 8 AND 9. LIPOIDS IN THE MUCOSA OF ADULT HUMAN GALL-BLADDERS REMOVED BY SURGICAL OPERATION (SCHMIEDEN'S CLINIC)

Figure 8 is a longitudinal section from the body of a gall-bladder removed from a 27-year-old female patient (× 80). A fold of the mucosa is shown which is bigger and plumper than usual. In sections stained for fat the wide spaces representing the bodies of large cells with an excentric small nucleus (endothelial leucocytes) are found loaded with anisotropic lipoids. The gall-bladder did not contain biliary calculi. Although characteristic signs and symptoms of gall-bladder disease had indicated surgical intervention, no other changes were found besides the demonstrable lipoid content of the mucosa.

Figure 9 is a longitudinal section from the neck of the gall-bladder removed from a 23-year-old female patient (× 30). The anisotropic lipoids in the subepithelial layer are represented by intensively dark areas. Biliary calculi were not present. Although characteristic signs and symptoms of gall-bladder disease had indicated surgical intervention, no other changes were found besides the demonstrable lipoid content of the mucosa and a slight cellular infiltration of the subepithelial layer and the intermuscular septa.

FIG. 10 LONGITUDINAL SECTION FROM THE FUNDUS OF AN OVERDISTENDED GALL-BLADDER FROM A FEMALE INFANT, 9 MONTHS OLD (DEAD FROM MILIARY TUBERCULOSIS). × 20

The gall-bladder was overdistended by immediate post-mortem injection of formalin. The folds of the mucosa seem in many places to have disappeared entirely. The muscularis is very thin, and due to secondary injection the perimuscularis is oedematous and shows tremendously dilated lymphatics.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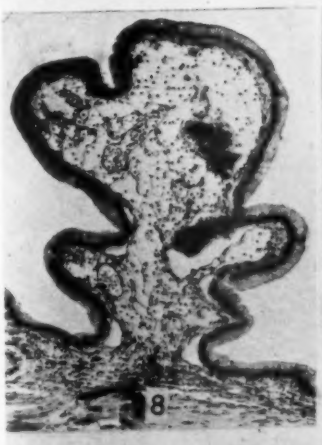
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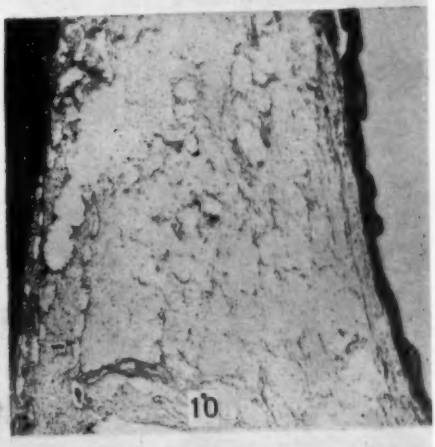
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bundles. The arrangement of the muscle bundles in an inner more or less longitudinal and an outer, rather oblique or circular layer, may help in distinguishing between a longitudinal and a transverse section. Another landmark which may guide in such a differentiation is the arrangement of the folds. In a transverse section, especially if taken from the proximal half (i.e., towards the neck) of the gall-bladder, higher folds are more numerous and lie closer to one another; in a longitudinal section, on the other hand, the folds are less numerous, are lower and found further away from one another.

The thickness of the muscularis, depending, of course, upon the development of the individual muscle bundles, varies considerably in different gall-bladders and also in different parts of the same gall-bladder. This becomes evident when sections (both transverse and longitudinal) from various parts of the same gall-bladder are compared with one another. A gradual decrease towards the fundus and a slight increase towards the collum are noted. Approaching the level of the first Heisterian fold, the muscle bundles usually decrease in number (compare Figures 11 and 12), and their arrangement into inner longitudinal and outer circular or oblique bundles seems to become disorganized. The muscularis gradually disappears as the cystic duct approaches the common hepatic duct. Usually a thin band of muscle fibers is found, however, in the base of each Heisterian fold. Between the web of the longitudinal, oblique or circular muscle bundles and the vessels which pass through this layer, a very scant cellular connective tissue is distributed. This intermuscular connective tissue forms a sheath around the muscle bundles and the vessels, filling out the space between them, and continues into the subepithelial layer on the one hand and into the perimuscular layer on the other. The amount of the intermuscular connective tissue is usually not

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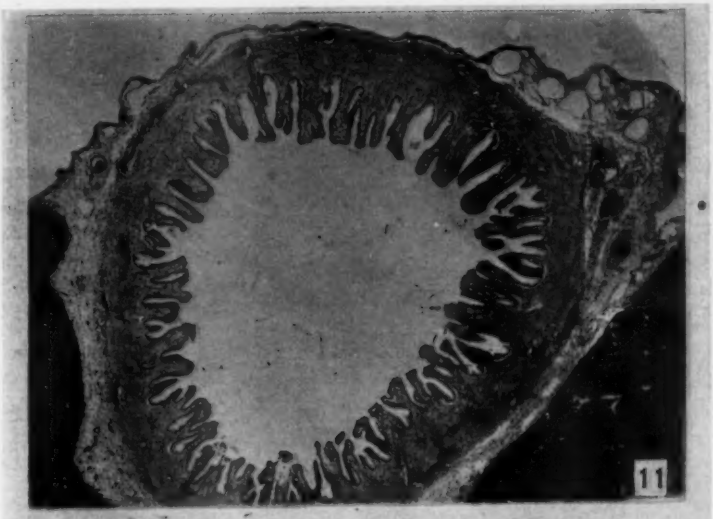
FIGS. 11 AND 12. TRANSVERSE SECTIONS FROM A CONTRACTED GALL-BLADDER OF A FEMALE INFANT, 3 MONTHS OLD (DEAD FROM GASTRO-ENTERITIS). × 20

The section shown in figure 11 is taken from the distal half of the gall-bladder near to the fundus, that shown in figure 12 from the neck about the level of the folds of Heister. Comparing the layers in these two sections, a marked difference is noted especially in the thickness of the muscular coat. No glands, nor penetrating outpouchings of the mucosa towards the external layers can be seen.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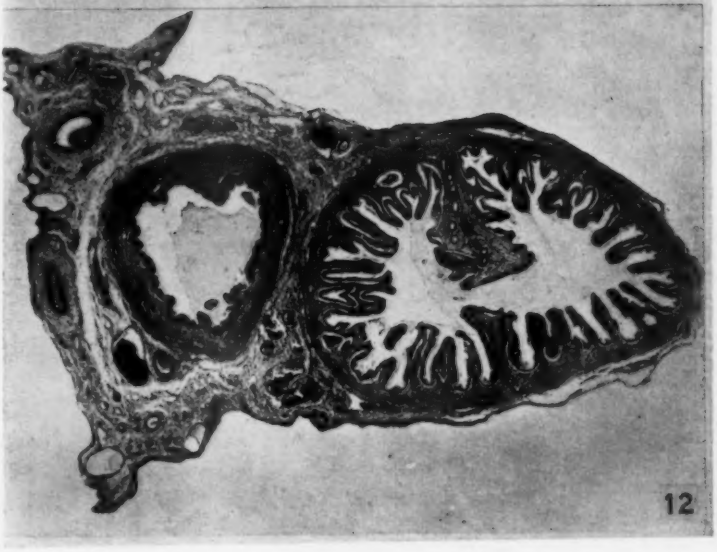
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considerable; a marked increase is noted under certain pathological conditions.

The muscularis is found to take part in modeling the surface relief of the mucosa. The influence is explained by the thinness of the lamina propria mucosae and the unevenness of the surface of the muscularis towards the mucosa. The connective-tissue layer between the epithelial lining and the muscularis is scant because the gall-bladder has no submucosa; the inner surface of the muscularis is uneven because the longitudinal muscle bundles are distributed mostly beneath the mucosa and because they do not form a compact layer. Under normal conditions, i.e., when the gall-bladder is moderately distended, the influence of the muscularis upon the surface of the mucosa is hardely noticeable, but becomes evident in case of over-distention or extreme contraction of the viscus. In a moderately distended gall-bladder only the pronounced elevations and the deeper indentations of the muscularis are followed by the mucosa. In an extremely distended gall-bladder the folds are lower, more widely separated from one another and many of them disappear entirely (Fig. 10). While the gall-bladder is undergoing extreme contraction, the mucosa has to adapt itself to a smaller area of attachment; as the viscus diminishes in size the folds come closer to one another, become thicker and project more deeply into the lumen of the gall-bladder. Since the muscle bundles next to and beneath the mucosa do not form everywhere a compact layer, the mucosa may dip down between the muscle bundles in some places and protrude under certain conditions towards the external layers (Fig. 7). The sites for such protrusions are most favorable where there are small defects in the muscular coat. Such are found regularly where the vessels penetrate the muscularis to reach or leave the mucosa, but sometimes also independently. In the formation of outpouchings of the mucosa towards the external layers, i.e., the formation of the Rokitansky-Aschoff sinuses, these defects of the muscular coat are found most significant (Fig. 7). It must be said, however, that in the healthy human gall-bladder Rokitansky-Aschoff sinuses are very rarely seen (Figs. 10, 11, and 12). If present at all, they are shallow and usually do not penetrate the muscular coat.

It was found that sudden overdistention of the intact gall-bladder (Fig. 10) is not followed instantly by formation of Rokitansky-Aschoff sinuses nor does extreme contraction lead by itself to their formation (Fig. 11). Apparently under normal conditions, especially when the healthy gall-bladder is moderately distended, the muscle bundles lie close enough to one another to prevent deeper out-pouchings of the mucosa towards the external layers.

Based on the morphological findings in gall-bladders with numerous and typical Rokitansky-Aschoff sinuses it was concluded that, in the process of formation of deeper outpouchings of the gall-bladder mucosa towards the external layers, extreme contractions following prolonged and repeated overdistentions of the viscus are most likely to play the leading part: Overdistention (especially acute dilatation of the biliary vesicle), because it increases the size and possibly also the number of potential holes in the web of the muscularis, and extreme contractions because as the viscus diminishes in size, and the mucosa adapts itself to a smaller area of attachment, the mucosa may be forced in some places to dip down between the muscle bundles and protrude towards the external layers.

It is well to remember, however, that overdistention or extreme contraction of the gall-bladder rarely, if ever, occurs under normal conditions. There is ample evidence that the healthy gall-bladder of a living human being is never overdistended or extremely contracted, but is always in a state of moderate distention and is practically never found empty. In this connection it should only be mentioned that in some animals a contraction capable of bringing the mucosa of opposite sides into contact with one another is altogether impossible because of the structure or the topographical relationships of the gall-bladder. In the two-toed sloth (Choloepus Hoffmanni) for example, Dr. Wislocki and the writer found the muscularis on the liver surface of the gall-bladder missing in some places and scant in others as compared with the relatively large amount of musculature on the peritoneal surface. The latter, however, comprised only about one-third of the entire circumference of the viscus.[2] In most of the writer's specimens

[2] The sloths on which these observations were made were obtained at the Barro Colorado Island Laboratory, Panama Canal Zone.

of Macacus rhesus[3] about two-thirds of the entire surface of the gall-bladder is found imbedded in the liver. In some of the specimens the liver edge, thinning out gradually, covers more than two-thirds of the entire surface of the gall-bladder. The cat's gall-bladder is often of a peculiar shape, having two or more fundi (bilobed, threelobed, etc.) some of which may be lodged deep in the substance of the liver.[4] Each of the two lions which the writer had an opportunity to examine had bilobed gall-bladders. A somewhat similar arrangement was observed also in one of the twenty-five monkey gall-bladders. Undoubtedly, conditions such as those just mentioned seriously interfere with a completely evacuating contraction of the biliary vesicle. Instances are known in which the gall-bladder is totally imbedded in the liver; in these cases, of course, an emptying contraction of the viscus is *a priori* utterly impossible. The writer, however, has never seen a human case of this type. His two specimens of this kind are that of a rhesus monkey and that of a pig. The significance of this latter condition in reference to accessory lobes of the liver which lie near or on the gall-bladder and for aberrant bile ducts will be referred to in the chapter on the "true Luschka ducts."

The examples just cited together with other morphological and more recent experimental data (experiments with methylene blue on rabbits in coöperation with Dr. D. M. Gay and with lipiodol on cats with Dr. E. C. Hill) support rather than disprove the view that the healthy gall-bladder as a rule does not empty itself by muscular contraction (Hutchinson 1903, Auster and Crohn 1922, Sweet 1924, Halpert 1924, Demel and Brummelkamp 1924, and others) as was formerly the general belief. The amount of bile, if any, which leaves the gall-bladder through the cystic duct within twenty-four hours is under normal conditions so minimal that the organ can hardly be looked upon as a reservoir with the function of supplying concentrated bile whenever there is call for such in the intestine. To the clinical studies of Schoendube and Kalk, Katznelson and Reiman, and others

[3] The writer had the privilege through the courtesy of Dr. Petroff in the Trudeau Sanatorium to obtain the biliary system of 25 monkeys. A detailed study of these will appear later.

[4] Boyden found in his series of over 2500 cats an average of one in eight possessing some form of accessory gall-bladder.

as well as to the recent experimental studies of Chiray and Pavel, Boyden, Whitaker, Kodama (a co-worker with Graham and Copher of St. Louis), McMaster and Elman, and others expressing opposite views the writer will refer in a later communication.

The perimuscularis (3) is a layer of connective tissue around the muscularis. It is usually greater in extent and looser in its texture towards the liver than towards the free or peritoneal surface of the viscus. In the perimuscular layer just beneath the muscularis there occurs a rich plexus of medium-sized blood vessels (plexus perimuscularis). In the zone of this vascular plexus the perimuscularis appears somewhat denser than it does towards the periphery where it is loose, less cellular and often contains fat cells. The denseness of the perimuscularis immediately beneath the muscularis is due mainly to the abundance of white connective-tissue fibers arranged into bundles running more or less in a transverse direction. The white connective-tissue fibers found here are much thicker than those in the subepithelial layer. Aschoff called the dense part of the perimuscular layer the "tunica fibrosa externa." In inflammatory processes this zone containing the middle-sized vessels of the viscus proliferates most extensively (Aschoff). The "neuro-vascular bundle" formed by the cystic artery, cystic vein, lymphatics and fairly large nerves, runs usually straight across the curves of the cystic duct and the neck of the gall-bladder. The main branches then are distributed for the most part near the margin of the free surface along, and parallel to, the right and left borders of the fossa vesicae felleae. The main branches of the structures forming the "neuro-vascular bundle" and to some extent the trunks themselves are found on the free surface of the gall-bladder immediately beneath the serosa (plexus subserosus).

The *serosa* (4), an additional layer formed by the peritonaeum, is found of course only on the peritoneal aspect, i.e., on about half of the surface of the gall-bladder, the other half being attached to the liver and void of peritonaeum.

In summarizing the data on the structure of the normal human gall-bladder it may be said, that three layers form the wall of the gall-bladder proper: (1) the mucosa, (2) the muscularis and (3) the perimuscularis.

1. The *mucosa* consists of a single row of uniform columnar epithe-

lium with no cuticula on the free margins, no goblet cells or glands. The epithelium rests upon a scant layer of connective tissue (lamina propria mucosae) called the subepithelium, which accommodates the rich capillary plexus of the mucosa (plexus subepithelialis). The human gall-bladder has no submucosa.

2. The *muscularis* is a web of longitudinal, oblique and circular muscle bundles, with a small amount of cellular connective tissue between them. This intermuscular connective tissue, scant as it is, forms a sheath around the muscle bundles and the vessels which penetrate the muscular coat; the connective tissue continues into the subepithelial layer on the one hand and into the perimuscular layer on the other. The arrangement of the muscle bundles and the amount of connective tissue between them is significant for the formation of outpouchings of the mucosa towards the external layers, i.e., for the formation of the Rokitansky-Aschoff sinuses.

3. The *perimuscularis* is a layer of connective tissue around the muscularis. Usually it is greater in extent and looser in its texture towards the liver than towards the free or peritoneal surface of the viscus. A rich plexus of medium-sized blood vessels occurs in this layer just beneath the muscularis (plexus perimuscularis). In the zone of this vascular plexus the perimuscularis appears somewhat denser (Aschoff's tunica fibrosa externa) than towards the periphery, where it is loose, less cellular and often contains fat cells. The main branches of the structures forming the "neuro-vascular bundle" and to some extent the trunks themselves are found on the free surface of the viscus immediately beneath the serosa (plexus subserosus).

A fourth layer, the *serosa* (4) formed by peritonaeum is found, of course, only on the peritoneal aspect of the viscus.

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A STUDY OF THE PRE-DISEASE DIETS OF PATIENTS WITH PERNICIOUS ANAEMIA

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The interest in the specific dietetic treatment of pernicious anaemia arose from Mosenthal's (1) high protein and energy diet, and from Gibson and Howard's (2) application of the Whipple high-iron diet to this disease. These investigators noted more favorable nitrogen balances and blood improvement on such regimes. Others have confirmed their results. Of unusual interest are the improvements, both haematological and clinical, systematically produced in 45 patients by Minot and Murphy (3) by the use of a special diet, characterized by 200 grams of lightly cooked liver per day, other proteins of good biological value, low fat, low carbohydrate, and an abundance of vitamine-containing vegetables and fruits. This may be characterized at present as an embellished application of Whipple's high iron diet, features other than the glandular organs having been added on somewhat empirical grounds. The therapeutic value of the diet, as reported by Minot and Murphy, is so definite and constant as to bring the entire subject of nutrition in this disease to a sharp focus of interest.

From an etiological angle, a strong suggestion presents of the possible influence of the lifetime food habits of these patients. Is it possible that deficiencies or excesses of any type of food have had a bearing upon the pathogenesis of the disease? In order to form an opinion on this matter the writer has undertaken a detailed study of the pre-disease diets consumed by 26 patients over a period, especially, of ten years before becoming ill, with additional inquiries as to personal tastes in food, meal intervals, and other points of relevant interest. The information was gained in each case by somewhat arduous questioning of the patient and nearest associates.

The personal equation of the patient—his memory and willingness

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to coöperate—unquestionably makes the study even more difficult than the admittedly difficult problem of determining what a normal person has been accustomed to eat. The results are presented herewith, and discussed from an absolute and a relative standpoint.

Their appetites since becoming ill are described in their own words, as follows: "Remarkably good" in 1 case; "good" in 2 cases; "fairly good" in 3; "variable but generally good" in 1; "variable" in 4; "variable with my condition" in 1; "good except when in relapse" in 1; "capricious" in 1; "fair" in 1; "not very good" in 1; "not so good" in 2; "not good" in 4; "poor" in 3; "none" in 1 case. These may be reclassified as follows:

Good to fairly good	6
Variable	8
Fair to poor	11
Bad	1

It will be noted that in at least 20 of the cases, the appetite had been definitely affected since the onset of the anaemia. It is not impossible, therefore, that after that time their diets may have been variously abnormal.

THE FOOD HABITS OF 26 PATIENTS WITH PERNICIOUS ANAEMIA BEFORE BECOMING ILL, WITH SPECIAL REFERENCE TO THE TEN YEARS PRECEDING THE ILLNESS

(1) Of the 26, 20 had always lived in the province of their birth (Ontario) usually within a radius of 40 miles of their present home.

(2) Diarrhoea before beginning of illness: Five had experienced the severe diarrhoea, before the disease, which characterizes, in larger series, from 20 to 30 per cent of the cases of pernicious anaemia. It had varied from two months to one year in duration.

(3) Indigestion before the illness: In 15 there had been a definite indigestion, of variable character and duration. In 5 it was regarded as of minor importance. In 10 it was a very troublesome complaint. Among these 10, 2 described it as "gas pressure;" vomiting had occurred in 2 cases. "Biliousness," "heartburn," "sore mouth," "pain in the stomach," "nausea," and "migraine" were other descriptions. In most cases the symptoms had extended over three to four

TABLE I*

CASE	AVERAGE DAILY CONSUMPTION IN GRAMS			AVERAGE TOTAL DAILY CALORIES	DAILY AND OCCASIONAL SOURCES OF VITAMINES			REMARKS
	Protein	Fat	Carbohydrate		Vitamin A	Vitamin B	Vitamin C	
1	71.5	113.5	298.7	2,503	Daily: butter milk cream r. oats potatoes Occas: eggs tomatoes lettuce cheese cabbage apples oranges	same as A	Daily: milk cream potatoes Occas: tomatoes lettuce cabbage apples oranges	High in w. flour, pork, bacon, rice, butter
2	148.3	285.6	339.2	4,721	Daily: milk butter r. oats potatoes	Daily: milk bread r. oats potatoes apples Occas: prunes	Daily: milk potatoes apples	High in w. bread, pork, bacon, ham, butter, rice; low in milk
3	101.8	131.7	346.6	2,980	Daily: milk cream butter eggs potatoes carrots	Daily: milk cream bread eggs potatoes carrots	Daily: milk cream potatoes carrots	High in w. bread, bacon, rice

* The standards used are calculated from: Laboratory Handbook for Dietetics (Revised Ed.), by M. E. Rose. (The Macmillan Co.); Feeding the Family, by the same author; Nutrition and Clinical Dietetics, by Carter, Howe and Mason. (Lea and Febiger.)

TABLE I*—*Continued*

CASE	AVERAGE DAILY CONSUMPTION IN GRAMS			AVERAGE TOTAL DAILY CALORIES	DAILY AND OCCASIONAL SOURCES OF VITAMINES			REMARKS
	Protein	Fat	Carbohydrate		Vitamin A	Vitamin B	Vitamin C	
4	23.8	46.4	155.3	1,135	Daily: potatoes, oranges carrots cream, Occas: lettuce milk	Daily: carrots w. bread potatoes oranges cream apples Occas: milk grapefruit lettuce	Daily: cream carrots potatoes oranges apples Occas: milk grapefruit lettuce	High in w. bread, bacon, rice
5	74.7	113.0	308.5	2,550	Daily: milk butter eggs potatoes cream	Daily: milk bread eggs potatoes cream apples	Daily: milk potatoes cream apples	Very high in pork; virtually no fruit or vegetables
6	78.8	149.7	259.2	2,700	Daily: milk butter r. oats potatoes carrots peas Occas: tomatoes cabbage lettuce	Daily: milk bread r. oats potatoes carrots apples peas, Occas: tomatoes onions cabbage lettuce	Daily: milk potatoes cabbage apples peas, Occas: tomatoes onions carrots lettuce	High in w. bread, bacon, pork, butter
7	56.0	96.6	188.2	1,847	Daily: milk butter eggs potatoes carrots	Daily: milk bread eggs potatoes carrots	Daily: milk potatoes carrots	High in w. bread, butter

TABLE I*—*Continued*

CASE	AVERAGE DAILY CONSUMPTION IN GRAMS			AVERAGE TOTAL DAILY CALORIES	DAILY AND OCCASIONAL SOURCES OF VITAMINES			REMARKS
	Protein	Fat	Carbohydrate		Vitamin A	Vitamin B	Vitamin C	
8	62.6	122.8	256.0	2,380	Daily: eggs butter potatoes Occas: oranges	Daily: eggs bread apples potatoes Occas: oranges	Daily: apples potatoes Occas: oranges	High in w. bread; no milk
9	38.7	90.7	284.1	2,107	Daily: butter cream r. oats potatoes carrots eggs	Daily: same as A plus apples	Daily: cream potatoes carrots apples	High in w. bread, butter
10	61.7	140.0	168.3	2,181	Daily: cream eggs butter r. oats potatoes Occas: spinach tomatoes onions milk prunes lettuce	Daily: cream eggs bread r. oats apples potatoes Occas: spinach tomatoes milk onions prunes lettuce grapefruit	Daily: cream potatoes apples Occas: spinach tomatoes milk onions lettuce grapefruit	High in w. bread, pork, bacon; low in milk
11	54.0	134.7	269.0	2,505	Daily: butter milk potatoes carrots	Daily: bread milk potatoes carrots	Daily. milk potatoes carrots	

TABLE I*—*Continued*

CASE	AVERAGE DAILY CONSUMPTION IN GRAMS			AVERAGE TOTAL DAILY CALORIES	DAILY AND OCCASIONAL SOURCES OF VITAMINES			REMARKS
	Protein	Fat	Carbohydrate		Vitamin A	Vitamin B	Vitamin C	
12	52.0	106.6	208.0	2,000	Daily: butter eggs cream potatoes oats carrots lettuce	Daily: Same as A, plus apples	Daily: cream potatoes carrots lettuce apples	High in w. bread, butter
13	136.1	260.4	287.9	4,040	Daily: butter cream milk eggs potatoes carrots oranges oats Occas: cabbage bananas	Daily: bread cream milk eggs potatoes carrots oranges r. oats apples Occas: onions cabbage bananas grapefruit	Daily: cream milk potatoes carrots oranges apples Occas: onions cabbage bananas grapefruit	High in w. bread, pork products
14	37.4	79.1	241.9	1,830	Daily: butter cream r. oats potatoes carrots Occas.: milk tomatoes cabbage	Daily: bread cream r. oats potatoes carrots apples Occas.: milk tomatoes onion cabbage	Daily: cream potatoes carrots apples Occas.: milk tomatoes onions cabbage	High in w. bread

TABLE I*—*Continued*

CASE	AVERAGE DAILY CONSUMPTION IN GRAMS			AVERAGE TOTAL DAILY CALORIES	DAILY AND OCCASIONAL SOURCES OF VITAMINES			REMARKS
	Protein	Fat	Carbohydrate		Vitamin A	Vitamin B	Vitamin C	
15	94.1	178.8	343.0	3,340	Daily: oranges eggs butter oats milk cream potatoes carrots	Daily: oranges eggs r. oats milk cream potatoes bread apples	Daily: oranges milk cream potatoes apples	High in w. bread, butter
16	66.4	100.8	384.4	2,711	Daily: milk butter potatoes r. oats cheese Occas: carrots tomatoes	Daily: milk w. bread potatoes r. oats apples cheese Occas.: carrots tomatoes	Daily: milk potatoes apples, Occas.: carrots tomatoes	High in w. bread, candy
17	60.6	131.7	361.4	2,874	Daily: cream oats butter potatoes Occas.: eggs	Daily: cream oats bread potatoes apples Occas.: eggs	Daily: cream potatoes apples	High in w. bread
18	71.5	137.9	335.2	2,868	Daily: cream butter r. oats eggs potatoes carrots	Daily: cream bread r. oats eggs potatoes carrots apples	Daily: cream potatoes carrots apples	High in w. bread, pork

TABLE I*—*Continued*

CASE	AVERAGE DAILY CONSUMPTION IN GRAMS			AVERAGE TOTAL DAILY CALORIES	DAILY AND OCCASIONAL SOURCES OF VITAMINES			REMARKS
	Protein	Fat	Carbohydrate		Vitamin A	Vitamin B	Vitamin C	
19	69.5	132.5	274.8	2,570	Daily: eggs butter milk oats potatoes carrots Occas.: lettuce tomatoes	Daily: eggs bread milk r. oats potatoes carrots Occas.: lettuce tomatoes onions	Daily: milk potatoes carrots Occas.: lettuce tomatoes onions	High in w. bread
20	76.7	141.6	350.6	2,985	Daily: eggs butter r. oats milk potatoes Occas.: tomatoes peanuts cocoanuts almonds	Daily: eggs bread apples r. oats milk potatoes Occas.: tomatoes peanuts cocoanuts navy beans almonds	Daily: apple milk potatoes Occas.: tomatoes	High in w. bread
21	56.4	143.6	175.4	2,220	Daily: eggs milk butter carrots potatoes r. oats Occas.: oranges lettuce tomatoes	Daily: apples eggs milk bread carrots potatoes r. oats Occas.: oranges lettuce tomatoes	Daily: apples milk carrots potatoes Occas.: oranges lettuce tomatoes	High in w. bread

TABLE I*—*Continued*

CASE	AVERAGE DAILY CONSUMPTION IN GRAMS			AVERAGE TOTAL DAILY CALORIES	DAILY AND OCCASIONAL SOURCES OF VITAMINES			REMARKS
	Protein	Fat	Carbohydrate		Vitamin A	Vitamin B	Vitamin C	
22	80.5	183.4	375.2	3,474	Daily: milk cream butter eggs r. oats potatoes carrots	Daily: milk cream bread eggs r. oats potatoes carrots apples	Daily: milk cream potatoes carrots apples	High in butter
23	53.4	118.6	332.1	2,610	Daily: butter r. oats eggs milk potatoes	Daily: bread oats eggs milk potatoes	Daily: milk potatoes	
24	83.1	187.9	357.5	3,454	Daily: eggs butter milk potatoes cabbage carrots Occas.: tomatoes lettuce	Daily: eggs bread milk potatoes cabbage carrots apples Occas.: tomatoes lettuce onions	Daily: milk potatoes cabbage carrots apples Occas.: tomatoes lettuce onions	High in w. bread
25	72.3	157.3	261.1	2,750	Daily: oats butter potatoes eggs cream Occas.: lettuce carrots tomatoes cauliflower	Daily: oats w. bread potatoes eggs cream Occas.: onions apples lettuce	Daily: cream potatoes Occas.: onions apples lettuce carrots tomatoes	High in w. bread

TABLE I*—*Concluded*

CASE	AVERAGE DAILY CONSUMPTION IN GRAMS			AVERAGE TOTAL DAILY CALORIES	DAILY AND OCCASIONAL SOURCES OF VITAMINES			REMARKS
	Protein	Fat	Carbohydrate		Vitamin A	Vitamin B	Vitamin C	
26	65.2	145.8	269.1	2,640	Daily: milk eggs potatoes butter cream r. oats Occas.: oranges	Daily: milk eggs potatoes cream w. bread r. oats Occas.: oranges	Daily: milk cream potatoes Occas.: oranges	High in w. bread, candy

years, in 2 cases over ten years. A physician described his pre-disease vomiting as "frequent voluntary emesis just for the sake of feeling better" and not due to nausea. In these 10 cases one would naturally expect that these patients had probably lived on somewhat restricted diets because of the dyspepsia.

(4) "Have you always been regarded as a good feeder?" Replies: "Yes" in 14 cases; "normal appetite" in 1 case; "extra good" in 1; "Decidedly" in 1; "good" in 1; "moderately good" in 1; "fairly good" in 3 cases; "only moderate" in 1 case; "No" in 2 cases; one patient said "not greedy but steady." These replies, as well as the confirmation from nearest associates, indicate that the majority were accustomed to eat at least average amounts of food.

(5) "Have you always preferred any particular kind of food?" Eleven expressed no preferences. From the other 15 the following preferences were confessed: "Beans," "Bread and butter," "salt pork," "cabbage and turnips," "carbohydrates," "pork," "vegetables," "vegetables and fruit," "lemons and sour fruit," "white bread," "roasts," "sweets," "fat pork and fat beef," "sweet stuff," "pastry and butter."

(6) "Have you always had a dislike for any particular kinds of food?" Eighteen had no particular dislikes. Among the other 8 the following aversions were noted: "Fruit," "pork," "vegetables and milk," "cake

and pies," "cake and meats," "parsnips, beets, tomatoes, spinach and cucumbers," "sugar," "macaroni."

(7) Regularity of meals: In 17 they were regular and in 9 irregular in varying degrees.

(8) Extra feedings besides regular meals: Thirteen habitually ate between meals or on retiring, and 13 did not.

(9) Preference for food—fried, boiled or baked. Ten had no special preference; 9 preferred food fried; 4 baked; and 3 boiled.

(10) "Did you as a rule, take your meats rare, medium or well done?" One was indifferent; 8 preferred them rare; 5 medium; and 12 well-done.

(11) "Do you remember any change occurring in your tastes at any period of your life?" Twenty-one had always liked the same things. Among the other 5, one had turned against meat just after the illness began, one had always hated rice until she became ill, one had taken a dislike to sweets twenty-three years before her illness, one had turned against fruits and sugar since becoming ill; one had tired of sweets since becoming ill. It is noted that any alterations in general taste occurred after the onset of the illness, except in one case.

(12) "Have you spent much of your life in the sunshine?" Twelve had spent very little of their lives in the sunshine, while 14 had spent practically all their lives outdoors.

(13) "As an infant were you breast-fed or bottle-fed?" One was bottle-fed; 20 were breast-fed; 5 were uncertain.

The patients were then requested to furnish the examiner with a menu list to represent the average daily consumption during the ten years previous to their illness. The breakfast, the noon-meal and the evening meal were taken down roughly at first, as a skeleton. They were to be average meals such as the patient had been accustomed to eat. Then the exact quantities of such food articles consumed were described as carefully as possible, sometimes by linear measurements, sometimes by volume, sometimes by weights. Extra feedings were averaged and added to the daily total. The whole was submitted to the criticism of the wife, husband or other close associate, and any points at issue settled judicially.

Finally, a list of 141 different food items was carefully gone through. This list included all dairy products, all cereals, all nuts, vegetables,

fruits (fresh, dried and preserved), sugars, candies, condiments, meats, fowl, fish, sea-foods, cakes and beverages. The patient indicated, in each case, the frequency with which he ate it, and the amount eaten at one time.

A table of standards was then adapted and used. By a critical study of the diet records in each case, it was possible to form some conception of the total average daily calories, and their proportions in protein, fat and carbohydrate. These results are given in Table I, along with the chief sources of vitamines A, B and C, for each patient. This distribution of vitamine sources is made on an average day's food, and additional but occasional sources are indicated in the same column. In the final column certain striking features of the diets have been noted in certain cases.

COMMENTS

(1) From reference to individual caloric requirements, it will be gathered that 13 men and 5 women were obviously undernourished, *provided they did not understate their diet.* This method of arriving at their food intake is so open to error that little emphasis on this point is justifiable.

(2) In the majority of cases, the diets were low in Vitamine C.

(3) It also seems that the mineral content might have been inadequate in most cases.

(4) The large amounts of white bread consumed were striking, but not peculiar to these individuals.

(5) The proportion of fat calories is seemingly large. This point is the only one common to the series.

DISCUSSION

At the termination of this investigation, the writer feels that, on the whole, these individuals have consumed fairly average diets. Their diets in most cases were the same, over ten years, as those of their nearest associates, who did not develop pernicious anaemia. It is probable, therefore, that pernicious anaemia is not due, fundamentally, either to excess or deficiency of any type of food.

Whether diet plays a partial rôle in the etiology is still an unanswerable question. The one known, constant and constitutional pe-

culiarity of these individuals, *viz.*, achlorhydria, might, in some way not understood, be related to dietary factors. If such were the case, then the high fat intake (a constant but not a specific phenomenon) might be related to the constitutional peculiarity and play a partial causal rôle.

A study of the diets after the patients became ill might disclose factors of importance, but any such factors would necessarily assume a subordinate etiological position.

IMPRESSION

Persons who later developed pernicious anaemia did not have any specific differences in their food habits from their associates who remained free from the disease. It is not impossible, however, that high fat intake may coöperate with constitutional changes in the gastro-intestinal tract to play a partial rôle in the etiology.

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BOOK REVIEWS

Clinical Neurology for Practitioners of Medicine and Medical Students. Largely based on the book by Prof. Hans Curschmann. By E. A. STRECKER and M. K. MEYERS. 410 pp. $3.50. (*T. Blakiston's Son and Company.*)

The authors, after professing to offer the fundamentals of Clinical Neurology to the general practitioner, proceed to dispose of the basic questions of the anatomy and function of the central nervous system in a few short pages.

The efferent and afferent pathways are described, some evidently new anatomical facts being mentioned. The cerebellum is then discussed—in three brief paragraphs, in which a number of views are expressed which are difficult to correlate with those generally accepted. The anatomical review is not satisfactory.

There is an interesting chapter on the method of examination, in which many useful points are brought out, though no very clear system is followed.

The real interest of the book lies in the clinical description and discussion of the most important neurological conditions. Each disease is preceded by a table of the chief signs and symptoms; the essential points in the etiology and pathology are briefly noted, and there is an excellent summary of the treatment. Unfortunately, there are no diagrams or illustrations.

Taken altogether, the book is quite interesting and contains much valuable information.

A. T. M.

Elements of Surgical Diagnosis. By A. PEARCE GOULD, M.D., F.R.C.S. Sixth Edition. 739 pp. $5.00. (*Paul B. Hoeber, New York.*)

This sixth edition still maintains the high standard which has been recognized by the surgical profession since the first publication. The author has greatly simplified his book by separating the diagnosis of injuries from that of diseases. The introductory chapters deal with methods of diagnosis, followed by diagnosis of injuries and finally diagnosis of diseases. The radiographic illustrations are excellent and well selected, but if more numerous would add considerably to the text, especially as a manual for students. The entire field is thoroughly and lucidly elaborated without unnecessary detail both for injuries and diseases with the exception of those of the eye. As a reference work it deserves a very high position with those of its scope and can be heartily recommended to students and the profession.

W. D. N.

Infant Mortality and Its Causes. By ROBERT MORSE WOODBURY. 204 pp. $3.50. (*The Williams & Wilkins Co., Baltimore, 1926.*)

In this small volume, the author, formerly Director of Statistical Research, United States Children's Bureau, has collected a number of important papers on various phases of infant mortality. He shows in the first chapter the marked reduction in deaths of infants from gastro-intestinal diseases, in the birth registration of the United States, a decrease of 34.6 in six years, 1915–21. One is surprised to learn that there has been almost an equal reduction, 32.2, in the death rate from respiratory diseases in the same period. There has been a smaller decrease in infant mortality rates from nearly all causes of deaths.

The reduction referred to is more pronounced in the urban than in the rural areas and it is attributable to the better organization of child health activities in the cities.

The infant mortality rate is affected by the family income. When this is unduly small, the mother is more apt to be away from home before and after the baby's birth, to its great detriment. In similar fashion, the infant death rate is higher in crowded homes with insufficient air space. Apparently, the second child in the family has the best chance to survive and the child born to a mother between 25 and 30 years of age.

In typical city populations, the mortality for artificially fed infants is between 3 and 4 times as high as that among infants fed at the breast. There is a marked difference in the death rate among infants of various nationalities in this country. The infant mortality is lowest among the Jews and highest among the Poles and Portuguese, but whether these variations are due to inherent racial differences or are the result of environmental conditions is not determined.

In a final chapter, the factors which play a part in making the infant death rate in New Zealand, 40.2 in 1924, the lowest in the world are considered. Undoubtedly favorable climate, good housing conditions, and a high economic level have tended to lower the infant mortality. A large share, however, of the result is due to a well planned and general distribution of information pertaining to maternal and child health, through newspaper articles and pamphlets, and to the activities of the group of special nurses who reach in the infant welfare stations about half of all the babies born in New Zealand.

The volume contains material valuable alike to the statistician and to one engaged in child health activities.

J. H. M. K., JR.

Pernicious Anaemia, Leucaemia and Aplastic Anaemia. BY J. P. MCGOWAN. 116 pp. $3.00. (*Paul B. Hoeber, New York, 1927.*)

This book of 116 pages is not, as the title suggests, a treatise on the diseases mentioned. It is a study of leukemia of the fowl, and an attempt, by correlating some of its features with those of these diseases, to draw conclusions as to the nature of the morbid processes concerned. The first chapter gives a thorough descrip-

tion of leucosis of the fowl. The next four discuss the development of blood cells, amitosis in blood formation and its significance, and the megacaryocyte and its function. Then follows a discussion, based on the foregoing, of the pathological changes in fowl leukemia. The three chapters concluding the book attempt to connect these findings with the human diseases mentioned in the title. The points of comparison are emphasized and from them are developed. hypotheses, some of which are suggestive but not well supported. Several points of view are expressed which might be widely challenged. In Chapters VIII and IX fatty changes in the liver and other organs in anemia—the word "adiposity" is used to designate this condition—are attributed to the accumulation in the fat depots of the body of the lipoids from the destroyed red blood cells. In Chapters VII and X pernicious anemia and myeloid leukemia are presented as manifestations of the same pathological entity, each at one extreme, between which all gradations may be seen. No doubt cases can be found with morphological changes bridging the gap between them, but the wisdom of classing together, even morphologically, two conditions so entirely distinct clinically, and no doubt etiologically, is open to question.

Some features of a disease "thumps," said to be due to iron deficiency in suckling pigs, are also likened to those of pernicious anemia and aplastic anemia, and conclusions drawn from similarities.

When reading the book, one wishes that more attention had been given to English composition.

F. A. E.

Diseases of Women. By HARRY STURGEON CROSSEN, M.D., F.A.C.S. Sixth Edition. 1005 pp. $11.00. (*The C. V. Mosby Company, St. Louis, 1926.*)

The appearance of the sixth edition of this book is evidence of its continued popularity. The author has brought his subject matter so well up to date that all the new methods of importance used in gynecological diagnosis and therapy are now included. Although the size and comprehensiveness of this volume tend to make it somewhat unwieldy for the beginning student, nevertheless, it contains such a storehouse of invaluable information that it deserves a place on the bookshelves of all who are now practising or who intend to practise gynecology.

L. B.

Cavernous Sinus Thrombophlebitis and Allied Septic and Traumatic Lesions of the Basal Venous Sinuses. By ROY WELLS P. EAGLETON, M.D. 196 pp. $4.50. (*The Macmillan Company, New York, 1926.*)

This monograph is an important contribution to the literature and is a record of an unusually extensive experience with septic thrombosis of the cavernous sinus. The clinical material comprises reports of twenty-five cases with twenty-one deaths, one unknown outcome, and three recoveries.

The author stresses the need of making the diagnosis early and emphasizes three points: (1) that cavernous sinus thrombosis should be regarded as a group of

diseases; (2) that the classical symptoms may or may not be present; and (3) that in the cases of slow involvement without classical symptoms there are certain mechanical factors which determine the symptomatology. He divides the cases into two distinct clinical types: acute fulminating and chronic compensatory. The classical symptoms are found in the first type and are due to sudden and complete venous blockage.

Surgical treatment is discussed at length. The author advocates eradication of the primary focus, drainage of the cavernous sinus and ligation of the common or internal carotid artery, followed by a course of immunizing transfusions. His surgical approach to the cavernous sinus follows the path of invasion and he believes that drainage holds out a fair chance for recovery if undertaken before the sinus contains a suppurating clot. Ligation of the carotid artery is performed in order to stop its constant inpact within the cavernous sinus, and thereby to put the inflamed area of thrombosis at rest. This line of reasoning is not quite clear to the reviewer. Of the recoveries reported one followed drainage of the lateral sinus and jugular bulb and ligation of the carotid, and another, drainage of the cavernous sinus and ligation of the carotid. The literature reveals only one case previously cured by operation on the cavernous sinus itself, that reported by Bircher in 1893.

The typographical errors are few. There are twelve autopsy reports given in detail and a rather complete bibliography, including abstracts of all reported recoveries.

J. J. C.

Symbionticism and the Origin of Species. By IVAN E. WALLIN. 171 pp. $3.00. (*The Williams & Wilkins Co., Baltimore, 1927.*)

The author in the first four chapters of the book reviews the literature on mitochondria, laying special emphasis on his own work. He contends that by cultural methods he has conclusively demonstrated that the mitochondria are organisms of a "bacterial nature" which live symbiotically in the cells of practically all organisms.

In the next four chapters he describes numerous cases of symbiosis and arrives at the following conclusions: "Symbionticism offers a rational explanation for many of the variations in the morphology and physiology of plants and animals. When these variations are of sufficient magnitude and permanence they constitute new species. The fact that mitochondria are universally present in the cells of all organisms higher than the bacteria, that mitochondria are bacterial in nature and that microsymbiosis can determine morphologic and physiologic changes in organs and cells, can lead to no conclusion other than that *Symbionticism is a fundamental causative factor in the origin of species.*"

The remaining chapters, dealing with "symbionticism and heredity," and "development and organic evolution," are devoted to an attempt to strengthen the conclusions presented above.

The book contains some interesting suggestions, but the evidence presented in favor of the principal conclusions reached—that mitochondria are "bacterial in nature" and that symbiosis results in the production of new species—is not convincing.

S. O. M.

A Classification of the Tumors of the Glioma Group on a Histogenetic Basis with a Correlated Study of Prognosis. By PERCIVAL BAILEY and HARVEY CUSHING. Cloth. $5.00. Pp. 175, with 108 illustrations. (*J. B. Lippincott Company, Philadelphia.*)

If one wishes to read the record of a scholarly attempt to bring order out of chaos in a group of tumors, generally regarded as hopelessly confused, he may satisfy his desire admirably by spending a few hours with Bailey and Cushing's book on gliomata. An estimation of the value of their contribution is not difficult, since any enlightenment of the profession on this most important and most widely misunderstood subject is most welcome. We would not argue with the authors over the absolute accuracy of their detailed histogenetic studies and the correlation of their observations. Whether they have or have not succeeded in making a trustworthy classification of brain tumors of the glioma group on the basis of their derivation from definitely and accurately identified specific brain cells makes little difference. The important thing is that in attempting to do this they have used methods of study and arrived at concepts of brain tumors which not only appear to be correct but which form a basis for a simple understanding of a very complex subject. Bailey and Cushing have done for gliomata what Krompecher did for basal-cell epitheliomata. The importance of this piece of work is at once apparent to any one whose interest, inclination or duty brings him face to face with the problems of origin, diagnosis and prognosis of tumors belonging to this most perplexing group.

W. D. F.

Local Immunization. By PROFESSOR A. BESREDKA, Pasteur Institute, Paris. Edited and translated by Dr. Harry Plotz, Pasteur Institute, Paris. 181 pp. $3.00. (*The Williams & Wilkins Company, Baltimore, 1927.*)

Besredka's book, Local Immunization, contains four chapters which deal with anthrax, staphylococcic and streptococcic infections, dysentery and the typhoid fevers; in the fifth he gives his theories on the subject of immunity.

It would seem that an expression more appropriate than "local immunization" might have been employed in connection with the author's conceptions of immunity. The term, local immunity, as applied by Besredka to the skin, does not refer to the presence of an immunity in one area of skin as opposed to its absence in another, but indicates that the immunity exhibited against some infectious agents is dependent entirely upon the non-receptive state of certain cells—reticulo-endothelial—within the skin.

Besredka's conceptions concerning natural immunity, immunity acquired through infection, passive immunity, and immunity produced by means of vaccines of dead or attenuated bacteria are similar in character and are the result of experiments which seemed to indicate that susceptibility to some infectious agents is dependent upon the specific sensitivity of certain cells of the body. His conception of infection and actively acquired immunity, briefly, is as follows: An infectious agent upon entering the body finds specifically sensitive or receptive cells. The reaction resulting from the contact between these cells and the virus gives rise to a third substance which repels the phagocytes. Since the negative chemotaxis prevents the phagocytes from performing their usual duties, signs of infection or disease follow. Eventually, if the individual does not die, all receptive cells become desensitized or accustomed to the virus. Then no more repelling substance is formed, phagocytosis ensues, and recovery follows. During the process of phagocytosis the virus is destroyed, and antibodies and antivirus appear in the blood. The antibodies represent the excretory products of the phagocytes and "their function in immunity is, in reality, entirely secondary, or negative, in certain cases." The antivirus arises from the destruction of the virus and is the important factor in producing passive immunity in susceptible individuals receiving convalescent serum. In fact, "all the manifestations of immunity, whether natural or acquired, active or passive, are regarded as one process—that is, the interaction of the antivirus with the receptive cell."

Antivirus arises *in vitro* as well as *in vivo*. The fact that broth that has been seeded several times with staphylococci or with streptococci ceases to support the growth of these respective organisms led Besredka to believe that the failure of these organisms to grow in culture filtrates is due to the presence of a specific antivirus. This antivirus, when placed in contact with receptive cells, desensitizes them to such an extent that no reaction occurs when they some in contact with the virus.

Besredka's statements and ideas are bold and will undoubtedly be challenged by many investigators. Nevertheless, increased activity in the field of immunology will result from his work and ideas, and good will ultimately be derived from them. In the preface the author states his own views in regard to the value of his ideas set forth in the fifth chapter: "In it we will find the theory of local immunity as the truth appears to us today. For the present, it stimulates work and discussion. This is its merit. We are far from believing that it will stand for ever—it will surely go the way of its predecessors." There is no occasion for the reviewer to amplify the author's sincere and just criticism of his own theories.

T. M. R.

Anatomical Texts of the Earlier Middle Ages: A study in the transmission of culture with a revised Latin text of *Anatomia Cophonis* and translations of four texts. By George W. Corner, Professor of Anatomy in the University of Rochester. 112 pp. Bound in paper $1.00, in cloth $1.50. (*The Carnegie Institution of Washington, 1927.*)

"The anatomist, marching between the philosopher and the physician, has ever been their companion in the vanguard of man's struggle for truth and freedom through self-understanding. This is particularly true of the twelfth and thirteenth centuries, when Europe received again the Graeco-Roman learning preserved in Islam during Europe's Dark Ages. Mathematics and medicine, with the ancillary sciences, were the first to be taken over from the Orient and the first to be revised according to the scholastic method. In this movement the anatomists took a notable part in maintaining the continuity of thought and method." Dr. Corner has endeavored to trace the order and dates of composition of the mediaeval anatomies, their sources and their authorship. The period of these books is roughly coincident with that of the crusades.

It was in the Medical School at Salernum that medicine was awakened after the Dark Ages; this school was not a miraculous relic of ancient Greece but was built up from several cultural influences, the monasteries, the presence of strong Jewish communities and by a mingling of peoples in trade and warfare. Before the appearance of Constantine in 1070 no knowledge of anatomy existed in Europe. Constantine translated into Latin a multitude of books suitable for monastic schools. The Pantegni contains two books on anatomy forming a systematic description of the tissues and organs of the body, dependent of course on Galenic tradition and without any evidence of actual observation of the animal body or of experiment.

After the advent of the Constantinian translations the school of Salernum saw the appearance of a great amount of medical writing. The first Salernitan anatomical demonstration is also known as the Anatomy of Copho. Since there is no printed edition of this text that is free from obscurities or corruptions, Dr. Corner offers a reconstructed text made by a free use of all the known versions. An examination of the second Salernitan demonstration shows that it is arranged as a public discourse or lecture to accompany a dissection of a pig. The work opens with a formal introduction in the scholastic style, in which the various organs are classified in a somewhat rigidly schematic way according to their respective functions in mediating the three species of vital spirits. This prologue duly recited, the lecturer gives directions for killing the pig and arranging the carcass, and proceeds to conduct a systematic examination of the various organs, giving as he comes to each region a few words of guidance to the dissector, then a description of the region or organ, and finally mentions one or more diseases or lesions which may affect the part under examination. A careful study of the text reveals the fact that the Salernitan anatomist has drawn the prologue and all the formally descriptive parts of his discourse from the Pentegni. The two Salernitan Demonstrations are contemporaneous and the second is in part a specific criticism of the first. The third text, *Anatomia Mauri*, is a brief discourse also intended to accompany a dissection of a pig. These three little books give a lively picture of men teaching from the specimen and not from books alone—an unexpected thing in mediaeval anatomy.

The fourth work "Anatomia Magistri Nicolai Physici" differs widely from those already discussed. It is, in the first place, a systematic descriptive anatomy dealing with all parts of the body in logical sequence rather than in the order dictated by convenience of dissection. It purposes to describe the human body rather than the pig. Moreover, the subject matter is handled in a highly organized style with much space devoted to definition, classification and arrangement of the material.

The fifth text is a product of the early part of the thirteenth century. Dr. Corner found a hitherto unknown copy of this book which is two centuries older than the other copies and which must have been written within a generation or two of the author's lifetime. Whenever composed and whoever the author, this little compendium of anatomy is one of the very first books of the Middle Ages to show the influence of Aristotelian biology. In this sense it marks one of the turning points in the history of thought.

Dr. Corner gives an entertaining discussion of the authorship, period and content of these five anatomical texts: a charming translation of portions of four of them is included at the end of the volume. The whole volume shows scholarship of a high order, and forms not only a fascinating presentation but also a splendid addition to our existing knowledge of the beginning of modern anatomy. It is a contribution of great merit—an example of research achievement into the obscure origins of present-day learning. The book is well worth the reading both for those interested in the History of Medicine and for those eager for the more cultural aspects of medical practice.

O. R. L.

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